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كلية الدراسات العليا

التاريـــخ: / /

نموذج رقم (16)
اقرار والتزام بالمعايير الأخلاقية والأمانة العلمية وقوانين الجامعة الأردنية وأنظمتها وتعليماتها لطلبة الدكتوراة

أنا الطالب: معاوية احمد صالح حداد الرقم الجامعي: (9040132)
تخصص: التغذية والتصنيع الغذائي الكليـــــــة: الزراعة

عنوان الاطروحة:

Microbiological Quality of Soft White Cheese Produced Traditionally in Jordan and Study of its use in the Production of Probiotic Soft White Cheese

الجودة الميكروبية للجبنة البيضاء الطرية المنتجة بشكل تقليدي في الاردن ودراسة استعمالها في انتاج جبنة بيضاء طرية غنية بالمحفزات الحيوية (جبنة بروبيوتيك).

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد اطروحات الدكتوراه عندما قمت شخصيا" باعداد اطروحتي وذلك بما ينسجم مع الأمانة العلمية وكافة المعايير الأخلاقية المتعارف عليها في كتابة الأطروحات العلمية. كما أنني أعلن بأن اطروحتي هذه غير منقولة أو مستلة من أطاريح أو كتب أو أبحاث أو أي منشورات علمية تم نشرها أو تخزينها في أي وسيلة اعلامية، وتأسيسا" على ما تقدم فانني أتحمل المسؤولية بأنواعها كافة فيما لو تبين غير ذلك بما فيه حق مجلس العمداء في الجامعة الأردنية بالغاء قرار منحي الدرجة العلمية التي حصلت عليها وسحب شهادة التخرج مني بعد صدورها دون أن يكون لي أي حق في التظلم أو الاعتراض أو الطعن بأي صورة كانت في القرار الصادر عن مجلس العمداء بهذا الصدد.

توقيع الطالب: .... التاريخ: ١٥/ ٨ / ٢٠١١

٢٠١١/٨/١٥

MICROBIOLOGICAL QUALITY OF SOFT WHITE CHEESE PRODUCED TRADITIONALLY IN JORDAN AND STUDY OF ITS USE IN THE PRODUCTION OF PROBIOTIC SOFT WHITE CHEESE

By

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This Thesis was Submitted in Partial Fulfillment of the Requirements for the Doctor of Philosophy Degree in Nutrition and Food Science

Faculty of Graduate Studies

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August, 2011

COMMITTEE DECISION

This Thesis/Dissertation (Microbiological Quality of Soft White Cheese Produced Traditionally in Jordan and Study of Its Use in The Production of Probiotic Soft White Cheese) was Successfully Defended and Approved on ----------------------

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DEDICATION
الاهداء

اهدي هذه الرسالة الى

والداي الذين لم يتوانوا عن دعمي و حثي على الصبر والايمان

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AKNOWLEDGEMENT

I would thank and owe my supervisor Dr. Mohammed I. Yamani for his help and support, as this work cannot be accomplished without his corporation and patience. And thanks also presented for the discussion committee prof. khalaf Al- Delaimy, Prof. Asem Al- Shihabi, Dr. Basem Al- Abdullah, Dr. Tariq Oasili for their considered additions.

I'd thank Prof. **Abdelrahim A. Hunaiti** , Prof. **Nabil Shawagfeh** Prof. **Hani Al-Dmour**, Prof. **Omar Kafawin** & Prof. **Mohammed Yamani** for their support during my study for the Ph.D.

I'd thank those who helped me on my research especially:

1. Eng. Rana Al-Akhras (food microbiology lab. Advisor)
2. Eng. Osama Al- Khatib – Nutridar Co.
3. Dr. Moez Al-Islam Faris- Petra University
4. Eng. Khalid Al-Said & Baker & yousef (Dairy factory)
5. Eng. Noor Al- Nababtih & Lama Abu- Hammour
6. Eng. Mohammad Bal'awi & Ezzat Tialakh- Tiba dairy factory
7. Eng. Mohammad Shahin- University of Jordan
8. Eng. Yaser Hamamdeh
9. Sana' Abu- Jabal- computer center

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ABSTRACT

To determine the microbiological quality of soft white cheese, thirty samples were collected from dairy selected workshops and plants in major Governorates in Jordan. Physicochemical analysis were also included in the testing. A questionnaire was filled by interviewing each producer about the quality, safety and processing aspects of the workshops or factories.

The averages of log_{10} of the standard plate count (SPC), lactic acid bacteria count (LABC), enterobacteriaceae count (EntC) and yeast and mold count (Y&MC) of the 30 samples were 8.3, 7.9, 5.4 and 3.0, respectively. A significant positive correlation (0.90) was noticed between SPC and LABC, indicating that most of SPC are LABC. *Staphylococcus aureus* count was < 10 CFU/g and *Salmonella* was absent in 25 g of the cheese. The averages of pH, acidity (% lactic acid), moisture and salt (NaCl %) in brine and in cheese and ash were 6.0, 0.53 %, 56.5 % , 12.6 %, 9.4, and 9.5, respectively. Fourteen of the samples gave positive results to the alkaline phosphatase test, denoting the probability of using unpasteurized milk in the production. The high microbial content of the cheese samples reflects generally the poor hygienic conditions during production and storage, lack of refrigeration and absence of heat treatment to eliminate microorganisms. It can be could be concluded from the questionnaires that there is a lack of standardized method for production and keeping of soft white cheese in Jordan.

Probiotic soft white cheese was produced from cows milk using *Lactobacillus acidophilus* and *Bifidobacterium lactis* and a combination of the two. The probiotic bacteria were propagated in commercial UHT skim milk and either added to the cow's milk used in the production before renneting or to the curd before pressing. After pressing and cutting, cheese pieces were kept in 7 % NaCl brine at 4 ^{o}C for 21 d.

The probiotic bacteria were still viable to the end of the experiment. Most of the cheese treatments still have probiotic numbers above10^6 CFU/g at one week (wk). Numbers at 2 wk were < 10^6 CFU/g. All probiotic cheeses that had the addition of probiotic to the milk remained above 10^5 CFU/g at the end of the experiment, where as most of those that had the addition to the curd dropped to a lower level. Generally, pH and salt (NaCl%) in the brine and cheese remained almost constant throughout the experiment (6.7, 6.5, respectively).

To overcome the drop in the final counts of the probiotic bacteria in the cheese, we recommend to increase the inocula of each probiotic bacteria by doubling the added amount of cultured skim milk. It was found that it is better to add the probiotic bacteria to the milk rather than to the curd, because the addition to the curd did not result in having higher numbers of probiotic bacteria in the product. Furthermore, the direct addition of probiotic bacteria to the milk would save time and effort, and it is more hygienic and ensures better distribution of the bacteria in cheese. All soft white cheese produced in this study were acceptable from sensorial point of view. Cheese produced using *B. lactis* in the milk was the most acceptable, followed by cheese produced using both *L. acidophilus* and *B. lactis* in the milk and control. Cheese produced using both *L. acidophilus* and *B. lactis* in the curd was the least acceptable but was not rejected by the panelists.

1. INTRODUCTION

The major cheese type produced in Jordan is the soft white cheese (jeben balady) (Haddadin, 2005). According to the Jordanian standards for soft cheese (JS 246: 2006), soft cheeses are defined as unripened soft cheeses ready to eat directly after processing. Its acidity should not exceed 1.9 % as lactic acid and should be kept under refrigeration.
In Jordan soft white cheese is usually produced seasonally from ewes or goat milk, or a combination of both, without addition of a starter culture or salt under unmechanized or artisanal conditions and is handled at various stages of manufacture. The method of production could be summarized in coagulating the milk for 40-60 min by rennet (Rennet is a natural complex of enzymes produced in any mammalian stomach to digest the mother's milk, and is often used in the production of cheese) after heating to about 35°C then pressing in cheese cloth. The cheese is usually directly consumed after production or used in the production of some Arabian confectioneries such as kunafeh. This soft white cheese has a very limited keeping ability even under refrigeration; various types of microorganisms may enter the cheese during manufacture and subsequent handling (Humeid and Tukan, 1986; Humeid *et al.*1990; Yamani *et al.,* 1987). A good portion of the soft white cheese produced seasonally in Jordan is used in the production of boiled white cheese (Herzallah, 1994).

A number of experimental studies have been executed on the Jordanian soft white cheese including meltability and stretchability (Abu-Alruz,*et al.,*2009), kinetic sensorial analysis (Haddadin, 2005), use of fungal rennin-like enzyme in the manufacturing (Tubesha , 2002), in-can heating preservation of nabulsi cheese with emphasis on halotolerant

bacteria' (Abu-Alruz , 1999), halophilic bacteria of cheese, and quality of cheese (Saleh,1998), the isolation of salt tolerant bacteria for use in the production of nabulsi cheese (Yamani *et al*.,1998), nabulsi a Jordanian cheese (Haddadin, and Shahin, 1995), the effect of pH and salt concentrations of the brine on the storage ability and sensory quality of boiled white cheese (Herzallah, 1994), in-bag steaming of white brined cheese as a method for preservation (Humeid *et al*., 1990), effect of the addition of yoghurt whey to cow's milk on curd formation (Abu-Ghoush,1992), and improving the traditional method of producing boiled brined white cheese (Humeid and Tukan, 1986).

With the growth of the functional foods area, a growing research interest has focused on the incorporation of probiotic bacteria such as *bifidobacteria* and some lactic acid bacteria into cultured dairy products to further enhance health and nutritional value of these products. These bacteria beneficially affect human health by improving the balance of intestinal microflora and improving mucosal defenses against pathogens, with other additional health benefits, which include enhanced immune response, reduction of serum cholesterol, vitamin synthesis and anti-carcinogenic activity (Boylston *et al.*, 2004).

Although considerable marketing and research attention has focused on fermented milks and yoghurts as food carriers for *bifidobacteria*, these products are not optimal for the maintenance of high concentrations of some strains, as evidenced by poor viability (particularly some strains of *Bifidobacterium*) in commercial yoghurts. An alternative means to maintain the viability of the *bifidobacteria* would be to incorporate the organisms into a product like cheese where the pH, lipid content, oxygen level, and storage conditions are more conducive to the long-term survival of *bifidobacteria* during processing and

digestion (Boylston *et al*., 2004).The biopreservative effect can be enhanced by the combination of probiotic strains with other lactic acid bacteria (Da Cruz *et al.*, 2009).

The objective of the study was to investigate the characteristics of the indigenous flora in the Jordanian soft white cheese. Such knowledge is needed to improve the technological production and to guarantee typical properties. The study also aimed to use the Jordanian soft white cheese as a suitable vehicle for probiotic bacteria, such as *Lactobacillus acidophilus* and *Bifidobacterium* spp., and developing a tasty original probiotic cheese based on the Jordanian soft white cheese.

2. LITERATURE REVIEW

2.1 Foreword

Cultured dairy products are an important part of the diet of many societies. These dairy products were initially developed as means to preserve milk, and they have desirable sensory characteristics (Boylston *et al.*, 2004). The fermentation by the microbial starter cultures preserves the product through the production of lactic acid from lactose and contributes to the development of characteristic flavor compounds. These products are now recognized for their nutritional benefits.

When cheese is produced following traditional procedures from raw milk, the environmental microflora play a fundamental role in fermentation and are one of the most important parameters affecting the cheese quality (Sengul, 2006). In addition, the biodiversity of bacteria involved in cheese production can be considered a fundamental factor for the maintenance of the typical features of traditional cheese products. Improved hygiene on the farm and at dairy plants, pasteurization of the cheese milk, precautions to prevent post-pasteurization contamination, the use of automated cheese making equipment and the use of a starter culture capable of producing nisin would reduce the number of non-starter bacteria in the cheese (Hayaloglua *et al*., 2002).

2.2 Soft white cheese

The manufacture of cheese is a form of milk preservation, as milk is highly perishable. All cheeses, whether rennet or acid set, can be classified as soft, semi-soft (semi-hard), hard, or very hard, depending on their moisture content. Although this

classification is arbitrary and impractical, it helps to systematically group together cheeses that are alike in certain basic features or characteristics (e.g., moisture content, as moisture determines the body, consistency or compactness of cheese). The production of cheese requires the coagulation of milk, in most cases through the action of chymosin on the K-casein steric stabilizing layer of the casein micelle. The rennet-induced milk coagulum is cut and homogenized to expel moisture in a process called syneresis. Curd is later drained, salted, and packaged into fresh cheese (Da Cruz *et al.*, 2009).

Soft white cheese is manufactured from ovine, bovine and/or goat milk or from mixtures of these milks (Fernandes, 2009). Soft cheeses may be manufactured from whole milk, skim milk, cream, whey or combinations thereof (Farkye and Vedamuthu, 2002). The cheese is traditionally made by adding 6- 20 % salt with or without the use of starter culture (Abdalla and Mohammed, 2010). The relatively high moisture content of soft cheeses makes them easily susceptible to microbial spoilage (Farkye and Vedamuthu, 2002).

The U.S. Code of Federal Regulations does not specify maximum moisture content for soft cheeses. However, a legal minimum of 50% milk fat in the solids phase (fat in dry matter, FDM) of the cheese is specified for soft ripened cheeses. Soft cheeses are defined as cheeses containing water in the fat-free cheese matter (Wff) greater than 61%, and fat in the dry matter, FDM of 10-50%. The FAO/WHO classifies soft cheese as cheese containing over 67% Wff. Data on the composition of various soft cheeses reveal moisture contents in the range of 50-80 % (Farkye and Vedamuthu, 2002).

2.2.1 Traditional soft white cheese

Jordanian soft white brined cheese is a very popular traditional dairy product produced by farmers or seasonal producers. Its main traditional production methods followed in Jordan are described by Humeid and Tukan (1986). It is produced without addition of starter cultures from raw or pasteurized milk. The most suitable characteristics of high quality cheese preferred by Jordanian consumer are parallel rectangular in shape with dimensions 8x4x1.5 cm, pure white, or yellowish in color if cows milk is used, should be compact, neither hard nor very soft easily broken by hand, free from holes, become elastic when heated, and free from rancid flavor (Abu-Ghoush, 1992).

Haddadin (1986) summarized many problems face white brined cheese production in Jordan. These problems are the non-uniform quality, flavor abnormality, gassiness and other textural problems which include sponginess or hardening at the end of the storage period.

2.2.2 Safety of soft white cheese and pasteurization of milk

Whilst the efficient pasteurization of milk (high temperature/short time; 72 ^{o}C for at least 15 s or low temperature/long time; 63 ^{o}C for at least 30 min) should eliminate the risk of viable vegetative pathogenic organisms, cheese can be made safely with raw milk. (Farkye and Vedamuthu, 2002). Many cheese makers use raw milk or add raw milk to the cheese milk, considering it essential for good flavour, primarily due to greater proteolysis and lipolysis by the raw milk microflora in the cheese. In the U.S., cheese manufactured from raw milk must be stored at a minimum of 1.7^{o}C for at least 60 days before consumption. This regulation limits the manufacture and sale of unripened soft cheeses from raw milk (Farkye and Vedamuthu, 2002).

Some cheeses are also made with thermized milk that has been given a sub-pasteurization temperature (57–68 °C for at least 15 s) designed to eliminate spoilage bacteria (European Commission (EC), 2005). For unpasteurized milk cheese, milk production is the first critical control point (CCP) in the cheesemaker's hazard analysis and critical control point (HACCP) plan (Robinson and Tamime, 2002).

Historically there have been outbreaks of infection associated with the consumption of cheese, and the predominant organisms responsible have included *Salmonella, Listeria monocytogenes*, verocytotoxin producing *Escherichia coli* (VTEC), and *Staphylococcus aureus* (Littlea *et al*., 2008). From the reported cheese-associated outbreaks of foodborne diseases, it is clear that cheeses made from unpasteurized or improperly pasteurized milks are the most frequent causes. The major cheeses involved in these outbreaks were soft surface-ripened cheeses or the low-acidity, unripened cheeses (Bintsis and Papademas, 2002). In Jordan neighboring countries cases of Malta fever (human brucellosis) have been associated with consumption of soft white cheese especially when seasonally produced (Al-as'ad, 2000).

Cheeses made from raw milk are particularly at risk since they may become contaminated by pathogens initially present in the milk. Pathogens may also enter cheese during processing, if hygiene and process controls are inadequate. In general, soft and semi-soft cheeses with high water activities present fewer barriers to pathogen survival and growth than do hard cheeses (Bintsis and Papademas, 2002; Fernandes, 2009). According to U.S. Food and Drug Administration, and EEC directive 92/46, the principal pathogens of

concern associated with milk and processed milk products are *Salmonella* spp., *L. monocytogenes*, *S. aureus*, pathogenic *E. coli* (Pacheco and Galindo , 2010). In 2006, the consumption of contaminated cheese accounted for the 0.4 % of the total foodborne outbreaks in Europe (European Food Safety Authority (EFSA), 2008).

Prevention of undesirable growth of microorganisms in cheese involves four steps: (1) Keep the microorganism out of milk or prevent its growth in milk (hygiene on the farm, quickly cooling the milk, short time between milking and cheese making). (2) Kill the bacteria (pasteurization). (3) Prevent contamination during manufacture of cheese (dairy plant hygiene). (4) Create an environment within the cheese so that if the microorganism is present, its growth will be limited (proper pH, salt, fermentation of all sugar, low storage temperature). However, the most universally accepted (but not always properly practiced) method of preventing defects caused by microorganisms is sanitation. In this regard, development of biofilms is important (Marth and Steele, 2001).

a. *Staphylococcus aureus*

Before the mid 1960s *S.aureus* was a common cause of cheese-associated food poisoning, for several reasons. *S. aureus* is able to tolerate salt and moderate acidity, and can multiply during cheese manufacture and ripening in soft cheeses. It may survive for long periods even in hard cheeses, and, if high enough populations are developed, enterotoxin may be produced, which persists for many months even after the cells have lost viability (Fernandes, 2009)

S. aureus was isolated from a Turkish White cheese, “Beyaz Peynir” during a ripening. This is a brined cheese variety with a soft or semi-hard texture and a salty and acid taste. In Lben (low pH and high moisture dairy product in Morocco), coagulase positive *S. aureus* was detected in numbers above 10^3 CFU/ml. High numbers of *S. aureus* are generally associated with the presence of toxin (>10^6 CFU/g of cheese or ml of whey). Cheese vats with low acidity for a long time might be a risk, because *S. aureus* might grow at a level high enough to produce the enterotoxins (Pacheco and Galindo, 2010).

b. *Salmonella*

There have been a number of large outbreaks of salmonellosis associated with cheese. For example, in 1976 in the US, pasteurized Cheddar cheese contaminated with *Salmonella Heidelberg* was linked to an outbreak of 28,000 - 36,000 cases of illness. In a further outbreak in 1982, in Canada, unpasteurised Cheddar cheese contaminated with *S. muenster* was implicated, and in this case the organism could still be detected after 125 days' storage. Another outbreak in Canada, involving Cheddar contaminated with *S. typhimurium*, occurred in 1984, and over 1,500 cases were confirmed. Both these outbreaks in Canada serve to illustrate the ability of *Salmonella* to survive for long periods in cheese at refrigeration temperatures. More recently, in 1996, an outbreak of *S. gold-coast* infection in the UK involved over 80 cases, and was associated with a Cheddar-type cheese. There have also been outbreaks linked to other cheese varieties, including Mozzarella (Fernandes, 2009). *S. typhi* can survive for 34 days in a domiati cheese with 50 g L^{-1}NaCl, but for only 16 days when the salt level was increased to 100 g L^{-1} (Bintsis and Papademas, 2002).

2.2.3 Microbiological quality

a. Standard plate count (SPC)

Determination of the presence of total bacteria count in milk and dairy products is important because it is used as a standard for sanitary and technological correctness of milk and the final product, as its quality too (Stojiljković, 2009). The numbers of total bacteria in Turkish white cheese 'Beyaz Peynir' produced from both salted milk and from curd salted was higher than those in cheese salted by brine immersion (Hayaloglu *et al*., 2002).

b. Lactic acid bacteria (LAB)

The important genera of LAB for dairy products are *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus* and *Pediococcus*. LAB may withstand a low pH because when grown in acidified medium, they maintain a higher pH in the cytoplasm than the culture medium. Higher internal pH is accomplished by pumping out protons (H+ ions) via ATPases. LAB are naturally present in raw milk and raw milk products. In traditional cheese making, they are transferred from old batch to new batch via whey addition. Selection of the beneficial natural flora in milk, such as *Lactobacilli*, *Streptococci* and *Lactococci* naturally occur in good batches and in many instances decrease the numbers of bacterial pathogens (Pacheco, and Galindo, 2010)

Lactic acid bacteria were likely the prime agents in producing soured (fermented) milk and dairy products. They are naturally present in the udder, and in the the raw milk of healthy animals and have spread in the dairy environment. Many species of LAB have the generally recognized as safe (GRAS) status and are extensively used in the manufacture of

dairy products. The prime property of LAB is the production of acids, especially lactic acid. The inhibitory capacity of this acid lies in its reduction of pH to levels below which bacteria can't initiate growth. Lactic acid has unique sensory qualities, a sour and pleasant taste and antimicrobial properties. It has the generally recognized as a safe (GRAS) status by FDA and EC (Pacheco, and Galindo, 2010).

c. Enterobacteriaceae

Enterobacteriaceae may usually cause spoilage by the production of internal gas in the cheese, resulting in slits, small holes, or blown packs. The problem can be effectively controlled by adequate hygiene and process control in manufacturing (Fernandes, 2009)

d. Yeast and mold

Yeast may cause spoilage of fresh cheeses (Fernandes, 2009), resulting in gas production and off-flavors and odors. It may also proliferate on the surface of ripened cheeses, especially if the surface becomes wet, causing slime formation. Yeasts most frequently isolated from spoiled cheese include *Candida* spp., *Yarrowia lipolytica*, *Pichia* spp., *Kluyveromyces marxianus*, *Geotrichum candidum* and *Debaryomyces hansenii.* The public health significance of yeasts in foods, including dairy products, has been considered by most health authorities to be very minimal. It has been suggested that spoilage yeast may have a role in protecting *L. monocytogenes* from acid in industrial type pilot cheese by metabolizing milk proteins, raising the pH, and potentially assimilating organic acids of cheese (Pacheco and Galindo, 2010).

Mold spoilage (Fernandes, 2009) is usually unpleasant in appearance, and may result in musty taints and odours. Molds are also responsible for liquefaction of the curd. There is also the possibility of mycotoxin production in some cases. Molds commonly involved in cheese spoilage include members of the genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Mucor*, *Fusarium*, *Monilia* and *Alternaria*. Effective hygiene is important in the control of mold spoilage in cheese, and rigorous cleaning procedures are needed to prevent the accumulation of mold spores. Filtered sterile air supplies, or ultraviolet light treatment may also be used to control contamination. The use of vacuum and modified atmosphere packaging helps to prevent mold growth on pre-packed cheese, but growth may still occur in residual air pockets or in packs that are improperly sealed or become punctured. Where permitted, antifungal agents such as sorbic acid or natamycin may be incorporated into packaging.

2.2.4 Effect of physical and chemical factors

a. Salt

Salt acts as a flavour enhancer and as a preservative, which permits the cheese to be held in the warm prevailing climate. Salt plays a multi-faceted role in cheese ripening and influences the physical, chemical, and biological properties of cheese. When cheese is placed in brine, NaCl molecules move from the brine into the cheese as a consequence of the osmotic pressure difference between the cheese moisture and the brine. Consequently, water in the cheese diffuses out through the cheese matrix so as to restore osmotic pressure equilibrium. After the cheese blocks have been removed from the brine, salt and moisture diffusion continues within the cheese. The rate of salt transportation from the brine into the cheese mass depends on the characteristics of the cheese (i.e., moisture and fat content,

acidity, surface area) and brining conditions (i.e., salt concentration, temperature, brining time, acidity of the brine) (Hayaloglu *et al*., 2002). Coagulation time increases with increasing sodium chloride concentration. The viability of probiotic bacteria being inversely proportional to salt concentration (Abdalla and Mohammed, 2010).

b. Other physical and chemical factors

The survival and growth of pathogens in cheese depend on many factors, including variations in pH, a_w, salts, the presence of competing microbiota, and the temperature and biochemical changes during ripening. Most of bacteria grow optimally at a pH close to neutrality. Acid curd cheeses might have a longer storage life and higher safety standards than neutral pH cheeses. A low pH decreases the growth rate of most of microbial pathogens and microbial spoilage. Among the pathogenic bacteria affected in their growth rate by the effect of low pH are species of the genus *Salmonella* spp., *L. monocytogenes*, *S. aureus,* and the pathogenic strains of *Escherichia coli* such as the enterohemorragic (ECEH), enteropathogenic *E. coli* (ECEP) enterotoxigenic *E. coli* (ECET), enteroinvasive *E. coli* (ECEI) and *E. coli* O157:H7. For instance, growth of *L. monocytogenes* has been reported at pH values ranging from 4.0 to 9.6. Lag phase and generation time increase considerably as pH decreases to below 6.5. *L. monocytogenes* can tolerate pH values up to 4.1 depending on other factors such as moisture content, storage temperature, and the presence of starter cultures. *E. coli* O157: H7 appear to be more acid-resistant, being able to withstand pH values as low as 3.0. Consequently, there is a potential risk that some VTEC strains, and especially strains of *E. coli* O157:H7 could survive the low pH associated with the cheese manufacturing process (Pacheco and Galindo, 2010).

Acidity of milk, cheese mass and brine during the production of white soft cheese should be monitored and kept under control, in order to prevent its enormous increase which would result in final product of bad characteristics. High pH values and high numbers of microorganisms in milk make good environment for intensive effect of proteolytic enzymes of lactic acid bacteria, micrococa, coliform bacteria, ferment and mildew, which would bring to deterioration of cheese (Stojiljković, 2009)

2.3 Probiotic dairy products

The word “probiotics” comes from two Greek words (προ and βιοτοσ) meaning “for life” and was initially used as an antonym of the word “antibiotic” (Wei-Yin Ng, 2009). Probiotics are defined as living microorganisms in foodstuffs which, when taken at certain levels in nutrition, provide equilibration of the intestinal flora, and hence have a positive effect on the health of the consumer. Probiotics are selected from the strains most beneficial for the host intestine that are bacteria from the genera *Bifidobacterium*, *Lactobacillus*, and yeast (*Saccharomyces*). Among criteria for microorganisms to be included in the probiotics group are 1) survival on passing through gastrointestinal tract at low pH and on contact with bile; 2) adhesion to intestinal epithelial cells; 3) stabilization of the intestinal microflora; 4) nonpathogenicity; 5) survival in foodstuffs and possibility for production of pharmacopoeia lyophilized preparations; 6) fast multiplication, with either permanent or temporary colonization of the gastrointestinal tract; and 7) generic specificity of probiotics (Tomasik and Tomasik, 2003).

There is enough clinical evidence to support certain health claims attributed to selected strains of *Lactobacillus* and *Bifidobacterium* spp. However, substantial work needs to be done to substantiate other potentially beneficial properties including immunomodulation, hypocholesterolemic and anticarcinogenic effects (Vasiljevic and Shah, 2008)

Probiotic cultures have been exploited extensively by the dairy industry as a tool for the development of novel functional products. Today, more than 100 probiotic-containing products, including pasteurized milk, ice cream, fermented milk, cheese, infant formula, fruit juices, single shots and oat-based products, are available worldwide (Wei-Yin Ng, 2009). Probiotics are also exclusively available in the form of powders, tablets, or capsules as dietary supplements, which are frequently produced by freeze drying method. The exposure of probiotics to freezing and dehydration may lead to cell injury and decreased viability during processing and storage. During processing, the temperature/phase changes and dehydration can lead to the damage of cell membranes, cell walls, ribosomes, and DNA. Moreover, membrane lipid oxidation may occur during storage. Therefore, by the time the product is consumed, viability of probiotics may be depleted to a level where the desirable health benefits cannot be provided. As capsules or tablets frequently contain low number of viable probiotic cells, incorporation of probiotics in foods, especially in dairy products, is more common. In fact, commercial dairy products fermented by probiotic cultures have had a history of safety. Among dairy products normally sold in the US, the most familiar is yogurt.

Some probiotics (especially *bifidobacteria*) are fastidious microorganisms, and are susceptible to environmental conditions, such as organic acid accumulation, high temperature, hyperosmotic pressure, low water activity, high redox potential (presence of oxygen) and the presence of inhibitory substances (Lourens-Hattingh and Viljoen, 2002). Consequently, survivability and stability of probiotics has become a marketing and technological challenge for the industry. The viability and activity of probiotics in the products have been frequently cited as a prerequisite for achieving numerous beneficial health benefits (Galdeano and Perdigón, 2004). Therefore, in order to obtain their desirable functional properties, probiotics need to be delivered in sufficient numbers with high viable rate (Godward *et al.,* 2000; Talwalker and Kailasapathy, 2004). A general agreement is that high dose is required, which range from 1 x 10^6 CFU/ml (Kurmann and Rasic, 1991; Tamime *et al*., 2005) to over 1 x 10^8 cfu/mL (Laurens-Hattingh and Viljoen, 2001). Kailasapathy and Chin (2000) suggested that, in general, the minimum therapeutic dose should be 1 x 10^8 to 1 x 10^{10} CFU/day. High dosage is likely required to compensate for the possible decline on the amount of the viable probiotic cells during processing and storage of probiotic product as well as passage through the upper and lower parts of the gastrointestinal tract.

Numerous studies were conducted in Asia, Australia, Europe and the US to determine the presence or viability of *Lactobacillus* and *Bifidobacterium* species (Lourens-Hattingh and Viljoen, 2002; Tamime, 2002; Weese, 2002; La Torre *et al*., 2003; Tharmaraj and Shah, 2003; Varga *et al*., 2003; Huys *et al*., 2006; Lin *et al*., 2006), and most showed the probiotic species were either misidentified or had low viability.

Various compositional and process factors significantly affect the viability of probiotic microorganisms in fermented milks including pH, titratable acidity, molecular oxygen, redox potential, hydrogen peroxide, bacteriocins, short chain fatty acids, flavoring agents, microbial competitions, packaging materials and packaging conditions, rate and proportion of inoculation, step-wise/stage-wise fermentation, micro-encapsulation, milk solid non-fat content, supplementation of milk with nutrients, heat treatment of milk, incubation temperature, storage temperature, carbonation, addition of salt, sugar and sweeteners, cooling rate of the product and scale of production (Champagne and Rastall, 2009; Mortazavian and Sohrabvandi, 2006; Tamime *et al*., 2005). Among mentioned, milk solid non-fat content, incubation temperature and final pH of fermentation possess remarkable impacts on viability of probiotics (Cruz *et al*., 2007; Mortazavian and Sohrabvandi, 2006).

2.3.1 Probiotic cheese

The unique aspects of cheese making could potentially enhance the viability of *bifidobacteria* in cheese. The lack of proteolytic activity in *bifidobacteria* slows their growth in milk (Shah, 2000). However, Para-k-casein and other casein hydrolysates produced by rennet activity during cheese making, as well as the proteolytic activity of the starter cultures, have been suggested as growth-promoting factors for *bifidobacteria* and may decrease the cultivation difficulties of *bifidobacteria* in cultured dairy products (Boylston *et al*., 2004).

Lactic acid bacteria (LAB) play a principal role in releasing specific compounds responsible for cheese flavor development. The LAB carry out the initial acidification of

the milk, which assists in gelation. In fact, its ability to produce acid rapidly is probably the most important property of starter bacteria. LAB also contributes to the proteolysis of cheese, as they can degrade the products derived from the rennet action on the casein (peptides of high and low molecular mass). In fact, some strains have proteinases associated with the cell wall, which preferably hydrolyze the casein, similar to endo- and exopeptidases. The free peptides of the casein are hydrolyzed to smaller peptides and free amino acids by the peptidases from the interior of the cells (Herreros *et al.*, 2003).

Cheese could provide a valuable alternative to fermented milk and yogurt as a food vehicle for probiotic delivery, due to certain potential advantages. It creates a buffer against the high acidic environment in the gastrointestinal tract, and thus creates a more favorable environment for probiotic survival throughout the gastric transit, due to higher pH. Moreover, the dense matrix and relatively high fat content of cheese may offer additional protection to probiotic bacteria in the stomach. Due to its manufacturing process, fresh cheese appears to be ideally suited to serve as a carrier for probiotic bacteria as it is an unripened cheese, during storage it is submitted to refrigeration temperatures, and its shelf life is rather limited. A number of scientific papers (Blanchette *et al.*, 1996; Sua´rez-Solı´s *et al.,* 2002; Buriti *et al.*, 2005b; Masuda *et al.*, 2005; Buriti, *et al.*, 2007) reporting the development of fresh cheeses containing recognized and potentially probiotic cultures have been published, which described suitable viable counts and a positive influence on the texture and sensorial properties of these cheeses.

The application of two-stage fermentation for cultured dairy products has been shown to be effective in increasing the viability of probiotic bacteria by allowing the

probiotic bacteria to become dominant prior to the addition of the starter cultures. Since starter lactic acid bacteria produce inhibitory substances against probiotic bacteria and grow faster than them during fermentation, the viability of probiotic bacteria could be reduced. Fermentation with probiotic bacteria initially for 2 h followed by fermentation with starter cultures may be helpful in improving the viability of the former and result in higher counts, as was reported for *L. acidophilus* 2409 and *B. longum* 1941 (Boylston *et al*., 2004).

There are many challenges associated with the addition of probiotics in cheese manufacture. An important one is maintaining the sensory characteristics of the cheese. Another challenge is the survival of bacteria in the cheese matrix during the production and ripening periods (Fortin *et al*., 2011)

Some key characteristics of cheese making that are relevant to the inclusion of probiotics are as follows: (1) Relative low moisture content; (2) Presence of salt (i.e. salt in moisture ratio); (3) Starter culture organisms (e.g. acid production, flavour production during the maturation stage and competition for nutrients); (4) Extended storage over 3 months, which can influence the biochemical activities, alteration of redox potential and re-organisation of the cheese structure (Tamime *et al*.,2005).

Various cheese varieties have been successfully used as carriers of probiotic microorganisms. Studies have demonstrated that Turkish white brined, Feta-type, Cheddar, Philippine white soft, Edam, Emmental, Domiati, Ras, soft, Herrgård cheeses, Quarg, and cheese-based dips compare favourably with yoghurt as delivery systems for viable probiotic micro-organisms (Ghoddusi & Robinson, 1996; Murad *et al*., 1998; Gardiner *et*

al., 1999; Barraquio *et al*., 2001; Osman and Abbas, 2001; Psomas *et al*., 2001; Ahola *et al*., 2002; Antonsson *et al*., 2002; Mehanna *et al*., 2002; Abdou *et al*., 2003; El-Kholy *et al*., 2003; Kasimoglu *et al*., 2004; Medici *et al*., 2004; Milanovic *et al*., 2004; Tharmaraj and Shah, 2004; Tungjaroenchai *et al*., 2004; Weinrichter *et al*., 2004; Yilmaztekin *et al*., 2004).

The successful development of probiotic-containing cheeses would provide cheese industries with a competitive advantage over existing products, which contributes to the expansion of the range of dairy products possessing better nutritional and physiological qualities (Boylston *et al*., 2004).

3. MATERIALS AND METHODS

3.1 Sampling

Thirty cheese samples were collected between Jul and Aug of 2010 from major Governorates (Amman, Al-Balqa, Jerash and Madaba) in Jordan using suitable icebox containing ice slush. Pre-sterilized forceps wrapped with foil was used to take samples of soft white cheese from each determined site in the Governorates, and was put in pre-sterilized sealable plastic bags at 500g from each site. Table 1 shows the places and dates of sampling sites. A questionnaire for information collection about the cheese was filled at the time of sampling by interviewing the cheese producers.

3.2 Microbiological tests

A pre-sterilized cheese knife was used to take 25 g of each sample taken from three different pieces avoiding the surface into presterilized bag, and 225 ml of 2% sodium citrate dilution buffer at 40-45 ^{o}C was added. The bag was placed and locked into a stomacher (Interscience bag Mixer, Germany) and mixed for 2 min. Appropriate serial dilutions were made from 10^{-2} to $10^{-7}$by aseptically pipeting mixed sample (Autopipet, Eppendorf, Europe) into 0.1% peptone water (Oxoid, UK) sterilized at 120±1^{o}C for 15 min as diluents for all microbiological tests except *S. aureus* where phosphate buffer was used (add 1.25ml of stock phosphate solution and continue to one liter) (Davis and Hickey, 2004; Laird *et al*., 2004). The pH was adjusted to 7.2 with sodium hydroxide, and was made up to 1 liter with distilled water. Then it was placed in small vials, sterilized at 121^{o}C for 15 min, and stored at 0 to 4.0 ^{o}C).

Table 1. The places and dates of sampling

Sample code	Place	Date	Sample code	Place	Date
B1	Al-Balqa - Jameel cheeses	23/6/2010	A4	Amman - Al-juniedi cheeses	14/7/2010
B2	Al-Balqa - Hani cheeses	23/6/2010	A5	Amman - Hammoudeh cheeses	16/8/2010
B3	Al-Balqa - Abu-Hammour cheeses	23/6/2010	A6	Amman – Al Bashaeir	16/8/2010
B4	Al-Balqa - Al-Marmouri cheeses	23/6/2010	A7	Amman- Alshami cheeses	16/8/2010
B5	Al-Balqa - Fahid cheeses	23/6/2010	A8	Amman- Carrefour- Al Mazra'a Katayef cheese	26/8/2010
B6	Al-Balqa - Hisba 1 cheeses	5/7/2010	A9	Amman- Al-MIzra Hilwa mist- Katayef cheese	26/8/2010
B7	Al-Balqa -Hisba 1 – goat cheeses	5/7/2010	A10	Amman – Al-Morouj cheeses- Katayef cheese	26/8/2010
B8	Al-Balqa - Hisba 2-a cheeses	5/7/2010	A11	Amman – Tiba food station – Al- sultan Katayef cheese	26/8/2010
B9	Al-Balqa - Hisba 2-b cheeses	5/7/2010	A12	Amman- Suileh- Al-Aizariah cheeses	26/8/2010
M1	Madaba - Ziad Abu-rukba cheeses	21/7/2010	A13	Amman – Al-sha'ab cheeses Katayef cheeses	26/8/2010
M2	Madaba - Hussein Abu-rukba cheeses	21/7/2010	J1	Jerash – Jersa cheeses	11/8/2010
M3	Madaba - Abu-Radwan cheeses	21/7/2010	J2	Jerash – Al-Haris cheeses	11/8/2010
A1	Amman -University of Jordan Dairy processing factory	14/7/2010	J3	Jerash- Al – Rayyan cheeses	11/8/2010
A2	Amman -Baladna cheeses	14/7/2010	J4	Jerash – Al-Fadi cheeses	11/8/2010
A3	Amman - Maha cheeses	14/7/2010	J5	Jerash- Al- Aa'elate	11/8/2010

3.2.1 Standard plate count (SPC)

Standard plate count was executed according to Laird *et al*. (2004). Plate count agar (Himedia, India) was prepared and sterilized according to the manufacturer's directions. One ml of each sample dilution, which prepared in the previous step, was inoculated in pre-sterilized plastic petri. The medium was poured at 45^{o}C of about 12-15 ml for each plate above the sample dilution, and mixed gently until dispersed. The dishes were incubated at 32±1^{o}C for 48±3 h. The number was calculated and expressed as CFU/g cheese.

3.2.2 Lactic acid bacteria count (LABC)

Pour plating of sample dilutions were done by using pre-prepared sterilized MRS agar (Oxoid, UK) after cooling to 45±1^{o}C. Plates were incubated at 37±1^{o}C for 48±3 h in reduced aerobic conditions (put inside polyethylene plastic bag where the open end of the bag under the plates). Individual colonies were tested for catalase reaction, gram reaction (Gram stain kit from Delta lab, Spain), and cell morphology. Gram-positive, catalase-negative cocci, or rods were tentatively considered to be lactic acid bacteria. Count was reported as CFU/g (Frank and Yousef, 2004).

3.2.3 Yeast and mold count

Yeast and mold count was made according to Frank and Yousef (2004). Plate count agar (Himedia, India) was prepared, sterilized, and cooled to 45±1^{o}C as the manufacturer's directions. Two ml of antibiotic solution (prepared by suspending 500 mg each of chlortetracycline hydrochloride (Tetradar, Jordan) and chloramphenicol (Panreac, Spain) in 100 ml of sterile, phosphate buffered dilution) were added per 100 ml of the medium and mixed. Surface (spread) plating technique was used by spreading 0.1 ml of the sample or

sample dilution over pre-dried plates using a sterile, bent-glass rod. The plates were incubated at 25°C for 5 d.

3.2.4 *Staphylococcus aureus* count

Twenty-five g of sample and 225 ml of phosphate buffer dilution were stomached for 2 min. Serial decimal dilutions were prepared. One ml sample suspension was aseptically plated on three pre-dried plates of Baired Parker agar (BPA) medium (Oxoid, UK). Inoculums were spread over the surface of the agar plate using sterile spreading devices. Plates were retained in upright position until inoculum is absorbed by agar medium. Plates were inverted and incubated at 35 ±1°C for 45- 48 h. Coagulase production was tested by selecting more than one colony of each type counted. Number of colonies on set of triplicate plates was added and represented by colonies giving positive coagulase test and multiplied by sample dilution factor (Henning *et al*., 2004).

a. Coagulase testing

Suspected *S. aureus* colonies were transferred into small tubes containing 0.2-0.3 ml brain heart infusion broth (BHI) and emulsified thoroughly. Agar slant of suitable maintenance medium (e.g., trypticase soy agar (TSA) (Diffco, USA) was inoculated with loopful of BHI suspension (Himedia, India)). BHI culture suspension and slants were incubated for 18-24 h at 35°±1°C. Slant cultures were retained at room temperature for ancillary or repeated tests in case coagulase test results are questionable. Amount of 0.5 ml reconstituted coagulase plasma with EDTA (Liofilchem, Italy) was added to BHI culture and mixed thoroughly, incubated at 35°±1°C and examined periodically over 6-h period for clot formation. Only firm and complete clots that stay in place when tube is tilted or

inverted were considered positive for *S. aureus*. Partial clotting must be tested further (ancillary tests). All samples were compared with well identified coagulase positive *S. aureus* from University of Jordan hospital laboratories (positive control) (Henning *et al*., 2004).

b. Gram stain

Gram stain (Deltalab, Spain) was made of all suspected cultures and they were observed microscopically.

c. Ancillary tests for *S. aureus*

- Catalase test

A loop was scraped across the growth of several colonies from BPA (Oxoid, UK) plate and emulsified in a culture tube containing 0.5 ml of a 1% Tween-80 solution. Amount of 0.5 ml of 3% hydrogen peroxide (Sigma Aldrich) was added and loosely covered. Effervescence was an indication of a positive test. Positive and negative controls for this test were made for comparison, using a previous well-identified *S. aureus* (coagulase positive *S. aureus* from University of Jordan hospital laboratories and LAB isolated from dairy products of the university of Jordan dairy factory respectively) (Henning *et al*., 2004).

- Anaerobic utilization of glucose (Merck, Germany) and mannitol (GPR, England)

A tube of purple broth containing 0.5% glucose and a tube containing 0.5% mannitol were heavily inoculated with the isolate to be tested. Layered enough sterile mineral oil over the surface of the broth to create a height of at least 25-mm. positive (the previous well-identified *S. aureus*), negative (LAB), and blank media controls

(without any addition) were run simultaneously. The test samples were incubated for 5 d at $37^o \pm 1^oC$, then examined for the color change (Henning *et al*., 2004).

3.2.5 Enterobacteriaceae count

Enterobacteriaceae count was done as described in the International Standard, ISO 21528-2: 2004. Pour plate technique was applied using violet red bile glucose agar (VRBG) (Oxoid, UK). Plates were incubated at 37^oC for 24 h±2h. Colonies are pink to red or purple (with or without precipitation haloes). After counting, five such colonies were chosen at random for sub-culturing of the biochemical confirmation tests. Each of the colonies selected for confirmation were streaked onto nutrient agar (Himedia, India) plates; the plates were incubated at 37^oC for 24 h±2h. A well-isolated colony was selected from each of the incubated plates for the biochemical confirmation tests.

a. confirmation tests

Enterobacteriaceae suspected colonies were confirmed by the following tests:

- Gram stain, (Deltalap, Spain).
- Oxidase test

Using a glass rod, a portion of each well-isolated colony was taken and streaked onto commercially available strips contains oxidase reagent (Oxoid, UK).

- Fermentation test

The colonies that gave a negative oxidase test were taken using a wire and stabbed into tubes containing glucose agar (Conda, Spain). The tubes were incubated at 37^oC for 24 h±2h.

Colonies that were oxidase-negative and glucose positive are confirmed as Enterobacteriaceae.

3.2.6 Presence of *Salmonella*

Twenty-five g sample were aseptically weighed into sterile stomacher bag. Amount of 225 ml sterile lactose broth were added and stomached for 2 min. This homogenate was returned to a sterile 250 ml bottle and mixed well. The bottle cap was loosened about 1/4 turn and incubated at 35°C for 24 ± 2 h. (International Standard, ISO 6785: 2001).

a. Isolation of *Salmonella*:

Isolation of *Salmonella* was accomplished according to the International Standard ISO 6785: 2001. Lid of incubated sample was tighten and shaken gently. Amount of 10 ml mixture was transferred to 100 ml selenite cysteine medium (CS) (Himedia, India).The mixture was vortexed and incubated for 18-24 h at 37 °C. Three mm loopful (10 µl) of incubated cultures were mixed and streaked on brilliant green agar(BG) (Himedia, India). Plates were incubated at 37°C for 20-24 h, then examined for the presence of colonies that may be *Salmonella*. If typical *Salmonella* colonies were absent, re-incubation for further 24 h would be performed. One to five colonies of typical suspected *Salmonella* were picked from each selective agar for biochemical tests.

b. Biochemical confirmation tests

One colony was picked with a sterile inoculating needle and slant TSI (triple sugar iron agar)(Himedia, India)was inoculated by streaking slant and stabbing butt. Without flaming, LIA (lysine iron agar)(Himedia, India), and urea agar (UA)(Himedia, India) slant were inoculated also, by stabbing butt twice and then streaking slant. TSI, LIA and UA

slants were incubated at 37°C for 24 h. Tubes were capped loosely to maintain aerobic conditions while incubating slants, to prevent excessive H_2S production (ISO 6785: 2001).

3.3 Physical and chemical tests

Sampling of cheese in the field was as mentioned at the first of this chapter. A representative sample that was mixed, stirred, and ground to make sample uniform, was taken for the following measurements (Hooi *et al*., 2004).

3.3.1 pH of cheese

A pH meter (HANNA instruments - USA) was used to measure the pH of fresh soft white cheese samples. It was used after calibration using buffers 4 and 7. The electrode directly immersed into a beaker containing well packed homogenized cheese so, the pH sensitive bulb and reference junction make good contact with cheese sample, and the sample temperature compensator was stabbed inside the cheese to correspond with it. The pH was read directly when the reading stabilizes (Hooi *et al*., 2004).

3.3.2 Titratable acidity (Lactic acid %)

Four g ground cheese were weighed in a small beaker; the sample was mixed gently but thoroughly with a stirring rod to become a paste (Hooi *et al*., 2004). Amount of 0.5 ml of phenolphthalein indicator (Fisher scientific co, USA) was added to the cheese sample, and then it was titrated with 0.1 N sodium hydroxide (ReAgent, UK)with mixing by the glass rod, to the first permanent color was changed to pink (30 seconds).Acidity was expressed as lactic acid (%) (1mL of 0.1 N sodium hydroxide (NaOH) = 0.009 grams of lactic acid). Acidity was calculated as follows:

$$\text{Acidity (lactic acid \%)} = \frac{\text{(ml NaOH) X (Normality of NaOH) X 9}}{\text{Sample weight in grams titrated}}$$

3.3.3 Moisture content

Moisture content was implemented as Case *et al*. (1985). Ten g of a blended homogenous sample of fresh white cheese were weighed in a pre-dried pre-weighed aluminum moisture dish on an analytical balance (ADAM- AFP, USA). The sample was distributed evenly over the bottom of the dish. The dish was placed on the metal shelf in the atmospheric oven (Binder, USA) at 105 ^{o}C for 16-18 h. The dish was removed from oven and cooled in a desiccator for at least 30 min or until the dish reached room temperature. Then it was weighed on analytical balance and the moisture was calculated from the following formula:

$$\text{\% Moisture} = \frac{\text{loss in weight x 100}}{\text{Weight of sample before drying}}$$

3.3.4 Salt (NaCl) in brine and cheese

Salt (NaCl) was determined as described by Hooi *et al*. (2004).

a. A representative sample, that has been previously mixed and ground to make sample uniform, was taken. Nine ml of the cheese brine was transferred by a clean pipette into a clean flask. The pipette was rinsed with an equal volume of distilled water into the same flask. Proceeded with step c.
b. Accurately, 10 g of fresh soft white cheese were weighed into 250 ml beaker. Fifteen ml of warm distilled water were added, and mixed to make a thin paste. Twenty-five ml of distilled water were added and mixed until the sample dispersed. The solution was transferred to 100 ml volumetric flask. The beaker was rinsed with distilled water several times and mixed thoroughly. Then it was filtered through

filter paper and until collecting approximately 50 ml of filtrate. Twenty-five ml were pipette in to a clean flask.

c. One ml of potassium chromate indicator (Laboratory Rasayan, India) - 10 % aqueous solution was added. After that, titration with 0.1 N silver nitrate (SUPERCHEM, England) was carried out, to the first pale red-brown color lasting 30 seconds. Sodium chloride was calculated as follows:

$$\% \text{ of sodium chloride (salt)} = \frac{\text{ml } AgNO_3 \text{ X N } AgNO_3 \text{ X } 0.0585 \text{ X } 100}{\text{Weight of sample or aliquot}}$$

Aliquot weight was determined by dividing sample weight by dilution and multiplying by volume pipetted.

3.3.5 Ash determination

Ash was determined according to Hooi *et al*. (2004). Accurately, 10 g of fresh soft white cheese samples were weighed in a prepared (cleaned by soaking in acid solution, dried and ignited) pre-weighed crucible. The weighed samples were evaporated to dryness in oven at 100^{o}C for about 1 h. The crucible was then transferred to the muffle furnace (Hermes electronics, Naber, Germany) regulated at 540 to 550^{o}C. Sample was incinerated until it is free of carbon and a light gray or white ash remains. The sample was removed from ash and cooled. Ash was moistened with a small amount of distilled water to form a paste and the ash particles were gently crushed with a flat-end stirring rod. To remove any adhering ash particles, the rod was rinsed with a few millimeters of distilled water and allowed rinsing to drain into the crucible. The sample solution crucible was returned to the muffle furnace and evaporated at lowered temperature of muffle 100^{o}-120^{o}C. The dried sample was ashed again for 1 h. The crucible was removed and cooled to room temperature in a desiccator until an ambient temperature was reached. Reweighed and the results was expressed as follows:

$$\% \text{ ash} = \frac{\text{weight of residue x 100}}{\text{weight of sample}}$$

3.3.6 Phosphatase test

Phosphatase enzyme was measured in fresh soft white cheese samples by using phosphatase kit test for milk and milk products as described by HEYL Company-Germany. Ten ml of sterilized distilled water was put in a sterilized test tube specified for each sample. Lactognost I and lactagnost II tablets were added to each test tube and crushed by clean glass rod. Cheese sample of 0.25 g was added to the previous mix and macerated in the solution. The tubes were incubated at 37^{o}C for 1 h. Lactognost III was added then and the color was observed. Positive and negative controls of phosphatase enzyme were performed by manufacturing fresh soft white cheese as described by Humeid and Tukan (1986), except that they were processed from raw milk without pasteurization. Cheese was then divided into two portions, the first one was boiled, which served as negative control, and the other was unboiled, which served as positive control. Subsequent, the above test of HEYL Company was applied to these two controls to ensure that they gave negative and positive results.

3.4 Probiotic cheese production

3.4.1 Bacteria cultures and propagation

Six probiotic cultures include *Bifidobacterium lactis* FD BB-12® (Chr. Hansen-Denmark); *L. acidophilus* HOWARU NCFM® (Danisco); *L. acidophilus* (ATCC 4356) (Microbiologics, UK); *B. lactis* HOWARU HN019 (Danisco); *B. breve* Yakult (Yakult Japan); and *B. lactis* from a supplement (Theralac probiotic master). From which two cultures were selected for application in cheese, one from *Lactobacillus acidophilus,* and the other from *Bifidobacterium* spp.

All strains were tested for purity by examining films from cultures stained by methylene blue staining (Loffler's methylene blue 0.002 %, as performed by Cowan and Steel, 1974) (Fisher Scientific co, USA). Cultures of *L. acidophilus* (beginning with 1% inoculum) were propagated weekly in a sterile 12% reconstituted skim milk (RSM) (Regilait, France) supplemented with 2% glucose and 1.2% yeast extract. Cultures were grown using 1% inoculum at 37°C for 18 h. *Bifidobacteria* were propagated using 1% inoculum in sterile RSM (Regilait, France) supplemented with 0.05% L-cysteine-hydrochloride monohydrate (Euro OTC pharma, GMPH) in order to provide anaerobic condition and to enhance their growth. Cultures were maintained in the same media at 4°C. Before enumeration, cultures were transferred successively three times for activation (Tharmaraj and Shah, 2003)

3.4.2 Culture cultivation

Pepton water diluent (0.01%) was prepared by dissolving 1 g of bacteriological peptone (Oxoid Ltd., UK) and 0.5 g L-cysteine-hydrochloride monohydrate (Euro OTC pharma, GMPH) in1 L of distilled water. Nine ml aliquots were autoclaved at 121°C for 15 min (Tharmaraj and Shah, 2003). DeMan rogosa sharpe (MRS) Agar (Oxoid, UK) supplemented with 0.5 g L^{-1} L-Cystein-HCL monohydrate (Euro OTC pharma GMPH),was prepared and autoclaved at 121 °C for 15 m. This medium was used for cultivation of the previous cultures. Cultures were incubated anaerobically at 37 ±1°C for 2 d. (Anaerbic Gen Gas-Pack 2.5L, Oxoid, UK). All the strains were also tested for purity using methylene blue (Fisher Scientific co, USA).

3.4.3 Intermediate medium for probiotic addition to cheese

UHT skim milk (Nadec, Saudi Arabia) of 0.1g fat/100ml and pH of 6.6 was used for cultivation and propagation of probiotic bacteria. Two presterelized 250 ml bottles were opened near the flam for the addition of 0.05% L-cysteine-HCL monohydrate. The bottles were filled with the UHT skim milk using a pre-sterilized 250 ml graduated cylinder and mixed thoroughly. Amount of 2.5 ml (1%) of the previous refrigerated cultures, which preserved on RSM, were added for the skim milk, and mixed thoroughly.

The inoculated bottles were incubated anaerobically at 37 ºC±1 for 2 d in jars using anaerobic Gen 2.5 L gas pack (Oxoid, UK). After 2 d, the skim milk was coagulated. Cultures were transferred to a new UHT skim milk successively three times for activation. The probiotic counts were determined for the bottles using MRS agar (Oxoid, UK) fortified with 0.05 % L-cysteine -HCl monohydrate. These cultures were then available for cheese fortification.

3.4.4 Effect of salt (NaCl %) in brine on the general acceptability of soft white cheese

An experiment of processing a small amount of soft white cheese (about three Kg) was conducted to determine salt concentration in brine used in keeping the cheese, which did not affect the taste of the cheese negatively. Soft white cheese was processed as described by Humeid and Tukan (1986). Different brine solutions (0 %, 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%wt/wt) were prepared using table salt (Sasi, Jordan). Nine of 500 ml cleaned jars were prepared for packing cheese and labeled with a different concentration (from 0 % to 16 %). Each jar took ca. 200 g cheese before the filling with each concentration of the brine solutions. The jars were left in refrigeration at 4 °C for about 2d.Twelve panelists were requested to choose the best suitable salt

concentration of cheese for them. The testing was conducted at the sensory evaluation laboratory-University of Jordan.

3.4.5 A preliminary experiment for dose determination of cultured skim milk

This experiment was conducted for estimation the amount of cultured skim milk needed to achieve at least 10^6 CFU/g of probiotic bacteria in cheese. A bottles of probiotic cultured skim milk of A, B, and AB (A: *Lactobacillus acidophilus,* B: *Bifidobacterium* lactis and AB: is a combination of the two bacteria) were prepared and counted for probiotic bacteria using MRS agar with 0.05% L- cysteine. Therefore, the amount of cultured skim milk to achieve 10^6 CFU/g or more was calculated for one Kg cheese curd. About 31 liter of milk of 1.030 specific gravity and 3.76 fat were processed to cheese as described by Humeid and Tukan (1986); before coagulation the milk was divided into approximately two equal portions one for *L. acidophilus* and the other for *B. lacis* probiotics. After coagulation, the curd was strained in a preweighed cheesecloth put inside a preweighed stainless steel sieve and squeezed by hand after wearing gloves until most of the whey was expelled out. Then the amount of curd was weighed. The suitable amount of a probiotic-counted cultured skim milk was determined in proportion to the curd weight, to reach the level of $\geq 10^6$ CFU/g. The amount of cultured skim milk was diluted with sterilized distilled water and distributed evenly on the surface of the cheese curd with mixing. After that, cheese making was continued as described by Humeid and Tukan (1986). The cheese was then distributed in jars with addition of 7% brine solution and were stored at 4 °C. After a one day, the probiotic cheese was counted for probiotic bacteria per g as indicated above using a homogenized, mixed, and ground sample. Using methylene blue the probiotic bacteria was diagnosed from cheese and cheese cultures for their distinctive morphology. The determined count was used as a guideline for the following probiotic cheese production.

3.4.6 Probiotic cheese production

Cheese was produced from pasteurized cow's milk as described by Humeid and Tukan (1986). This cheese was served as control. The following simple flow diagram (Figure 1) shows the description of the main steps of probiotic cheese production under hygienic conditions and utensils sterilization.

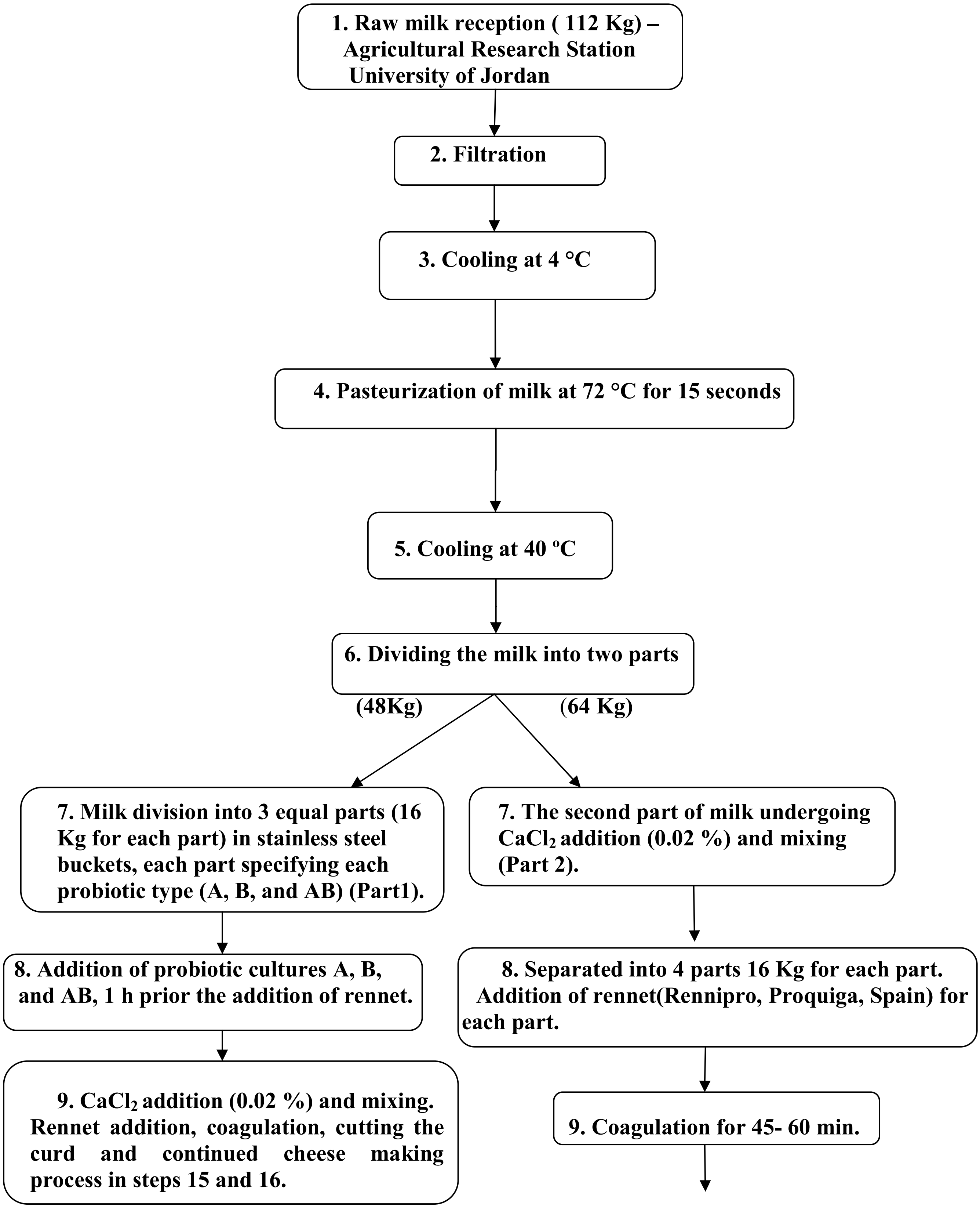
3.4.7 Testing of cheese

1. Milk was analyzed for pH, fat and specific gravity (Milk Analyzer, Milkotronic ltd, Bulgaria).
2. Samples of cheese from each manufactured batch were analyzed directly after packing (zero time) and at the assigned time intervals (2, 4, 7, 14, 21 d) during the shelf life of the product kept under refrigeration (4 ºC).Testing included the following which were tested as described previously:

- Probiotic bacteria count for treatments and control.
- Methylene blue staining
- pH for brine and cheese ((HANNA instruments - USA)
- NaCl % for brine and cheese (as indicted above)

3.4.8 Sensory evaluation of cheese

Sensory evaluation was carried out on samples of cheese collected after pressing and at the assigned time intervals from processing. Testing was included the control cheese also. Hedonic scale of 9 points, with five sensory attributes (appearance, smell, taste, consistency, and overall acceptability) was used for the evaluation of processed probiotic cheese of different culture treatments (Clark *et al*., 2009).


1. Raw milk reception (112 Kg) – Agricultural Research Station University of Jordan
2. Filtration
3. Cooling at 4 °C
4. Pasteurization of milk at 72 °C for 15 seconds
5. Cooling at 40 ºC
6. Dividing the milk into two parts
(48Kg)
(64 Kg)
7. Milk division into 3 equal parts (16 Kg for each part) in stainless steel buckets, each part specifying each probiotic type (A, B, and AB) (Part1).
7. The second part of milk undergoing $CaCl_2$ addition (0.02 %) and mixing (Part 2).
8. Addition of probiotic cultures A, B, and AB, 1 h prior the addition of rennet.
8. Separated into 4 parts 16 Kg for each part. Addition of rennet(Rennipro, Proquiga, Spain) for each part.
9. $CaCl_2$ addition (0.02 %) and mixing. Rennet addition, coagulation, cutting the curd and continued cheese making process in steps 15 and 16.
9. Coagulation for 45- 60 min.

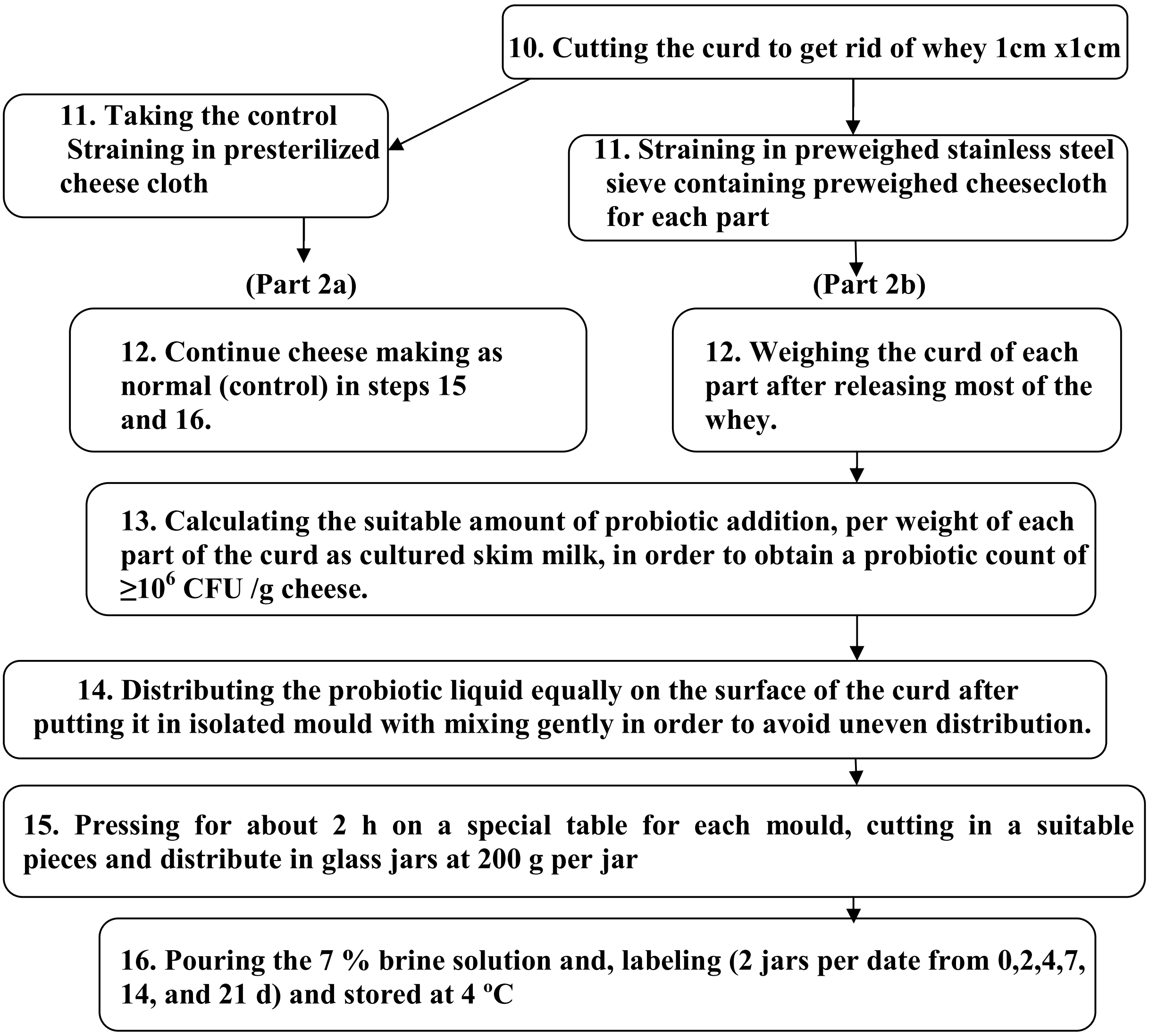


Figure 1. Flow chart of Jordanian probiotic cheese production main steps by addition of probiotic bacteria to the curd or milk.

3.5 Statistical analysis

Statistical analysis for microbiological levels, physical and chemical tests were analyzed using F-test, t- test (LSD), Tukey's Studentized Range and correlation of statistical analysis software (SAS) version 9; and table curve program version 5.1 for correlation regression analysis (SAS, 9; Delwiche and Slaughter, 2003).

4. RESULTS

4.1 Commercial Soft white cheese

4.1.1 Information about commercial production of soft white cheese

The questionnaire about soft white cheese collected from Jordanian Governorates (Al-Balqa, Madaba, Amman, and Jerash) in the period between 23/6 to 11/8/2010 provided with important information about processing and hygienic conditions of 20 soft white cheese processors in Jordan (small and large production scales). From investigating this questionnaire, we could conclude that the knowledge of cheese processors varied greatly. In addition, as observed from questionnaire, this industry differed from small scale and poor facilities to medium and large well-equipped facilities. The daily average of milk quantity used in processing of soft white cheese ranged between 100-500 Kg for small-scale producers to 300-5000 Kg for large-scale producers. Half of the 20 soft white cheese producers stop cheese processing when the sheep-milking period ends, all of them from the small-scale producers. According to the questionnaire most of soft white cheese producers used cows' milk for their production. Others used mixed milk of goat and cows or goat and sheep milk. The conversion ratio from milk to cheese for cow's milk, goat milk, cows and goat, sheep and goat, and sheep milk ranged between 5.5-9:1, 6-8:1, 6-8:1, 3-6:1, and 2.8-4:1 (Kg milk: Kg cheese) respectively. It was observed that only two of the 20 cheese producers used cooling tanks for milk transportation from the farm. Seven out of the 20 producers, most of them were from large-scale, used a pasteurizer for cheese production. The others used either stainless steel cookware (11 producers out of 20) or aluminum cookware (5 producers out of 20) or the two methods for milk pasteurization. The coagulation of milk was performed either in plastic pails (10 producers out of 20) or in

stainless steel tank (9 producers out of 20). One of the producers used the same aluminum cookware for pasteurization, for coagulation. Fifteen producers used stainless steel moulds for pressing the cheese, The rest of the producers used aluminum coated wood mould (2), aluminum mould (one), woody mould (one), porcelain mould (one). Temperature of rennet addition ranged between 30ºC to 38ºC.This range affects the conversion ratio. Chris-Hansen rennet was the most rennet used. Most of the producers dissolved the rennet in water either lukewarm or at room temperature before addition, except one who add the rennet on the milk on a dry state. The time for complete curd formation ranged between 20 min and 150 min which reflects the difference in processing conditions, specifications of cheese needed, and incubation temperature difference effect. Eleven out of 20 producers made cutting the curd, which affects also on the conversion ratio. Some of the producers add black cumin seeds as a tradition on the surface of curd. Others admitted that they add preservatives (e.g. Natamycin). Salt addition was mostly ranged between 16-18% wt/wt, and a few used between 10-14%. The shelf life ranged between 2- 4 wk. The questionnaire also shows that some small-scale processors add $CaCl_2$ and salt more than required for the processing of soft white cheese (60g/100 L milk, 20%, respectively). In addition, the pasteurization temperature varied between reasonable and scientific (72ºC/ 15s) to extra unneeded temperatures (100ºC/10 min). Half of the 20 processors workers that were studied did not wear the processing coat in addition the majority did not wear the head cover. Most of cheese producers recycled the cheesecloth for about 30 times until it began to tear. There were multiple methods used for cleaning cheesecloth such as boiling water, soda and Sar™ , and chlorine with hot water or steam.

4.2 Microbiological survey

Results of the microbiological survey of soft white cheese are shown in the following in details. Figure 2 shows the averages of Log_{10} of standard plate count (SPC) and counts of lactic acid bacteria (LABC), Enterobacteriaceae count (EntC) and yeast and mold count (Y&MC) of 30 soft white cheese samples collected from different Governorates.

4.2.1 Standard plate count (SPC)

Standard plate count ranged between 6.5 and 10.0 with an average of 8.3. It was found that there was no significant difference ($p > 0.05$) in SPC among the 30 cheese samples, and among different Governorates except between Amman and Al-Balqa. The averages of SPC for different Governorates were 8.1, 8.8, 8.1, 8.3 for Amman, Al-Balqa, Jerash, and Madaba, respectively.

4.2.2 Lactic acid bacteria count (LABC)

Lactic acid bacteria count ranged between 6.1 and 9.7 with an average of 7.9. It was seen that no significant difference ($p > 0.05$) was found in LABC among the 30 cheese samples and among different Governorates. The averages of LABC for different Governorates were 7.7, 8.2, 7.7, 8.2 for Amman, Al-Balqa, Jerash, and Madaba respectively.

4.2.3 Enterobacteriaceae count (EntC)

Enterobacteriaceae count ranged between less than 1.0 and 8.3 with an average of 5.5. There was no significant difference observed ($p > 0.05$) in EntC among the 30 cheese samples and among different Governorates. The averages of EntC for different

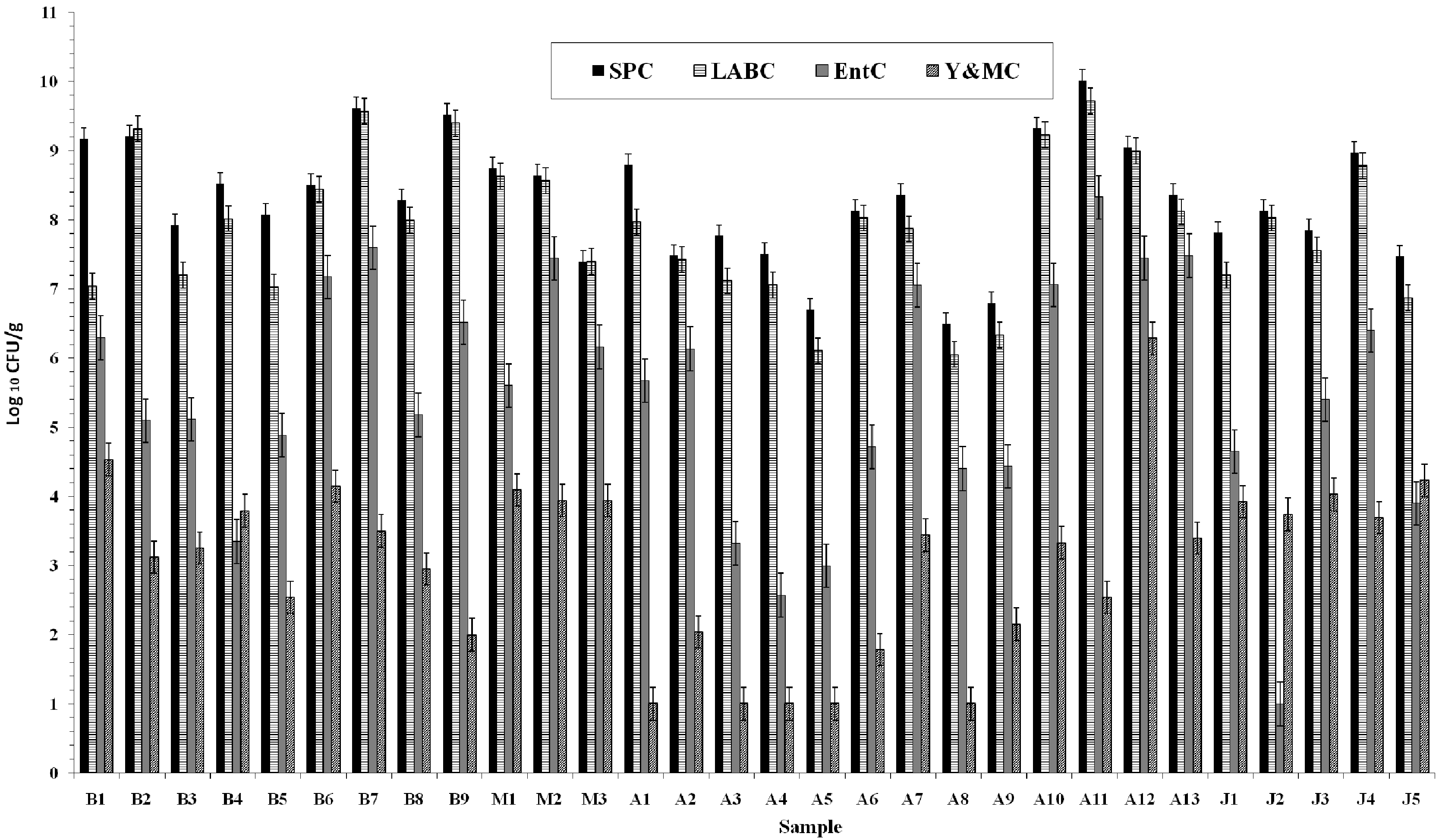


Figure 2. Standard plate count (SPC) and counts of Lactic acid bacteria (LABC), Enterobacteriaceae count (EntC) and Yeast and Mold count (Y&MC) of 30 soft white cheese samples collected from four different governorates in Jordan.
(B= Al-Balqa, M= Madaba,

Governorates were 5.5, 5.7, 4.3, and 6.4 for Amman, Al-Balqa, Jerash, and Madaba respectively.

4.2.4 Yeast and mold count (Y&MC)

Yeast and mold count ranged between 6.3 and less than 1.0 with an average of 3.0. There were significant differences observed ($p < 0.001$) in Y&MC among the 30 cheese samples and among Amman Governorate and each of Al-Balqa, Jerash, and Madaba. The averages of Y&MC for different Governorates were 2.3, 3.3, 3.9, and 4.0 for Amman, Al-Balqa, Jerash, and Madaba, respectively.

The highest and the lowest SPC, LABC, and Y&MC samples were seen in Amman Governorate. The highest EntC samples were seen in Amman Governorate also, but the lowest were seen in Jerash. A significant positive correlation (0.90) was noticed between SPC and LABC (Figure 3-A), which means that most of SPC are LABC. There was also a significant positive correlation between SPC and each of EntC and Y&MC (0.60, 0.39 respectively) (Figure 3-B and 3-C). A significant positive correlation between LABC and EntC (0.60) and Y&MC (0.36). (Figure 3-D and Figure 3-E).

4.2.5 *Staphylococcus aureus*

Only the colonies that gave positive results on all confirmed tests of *S. aureus* was considered as positive in cheese. All the 30 soft white cheese samples did not give positive results of *S. aureus* on all confirmed tests (Cagulase, gram stain, catalase, and anaerobic utilization of glucose and mannitol), therefore, it were documented as less than 10 CFU/g.

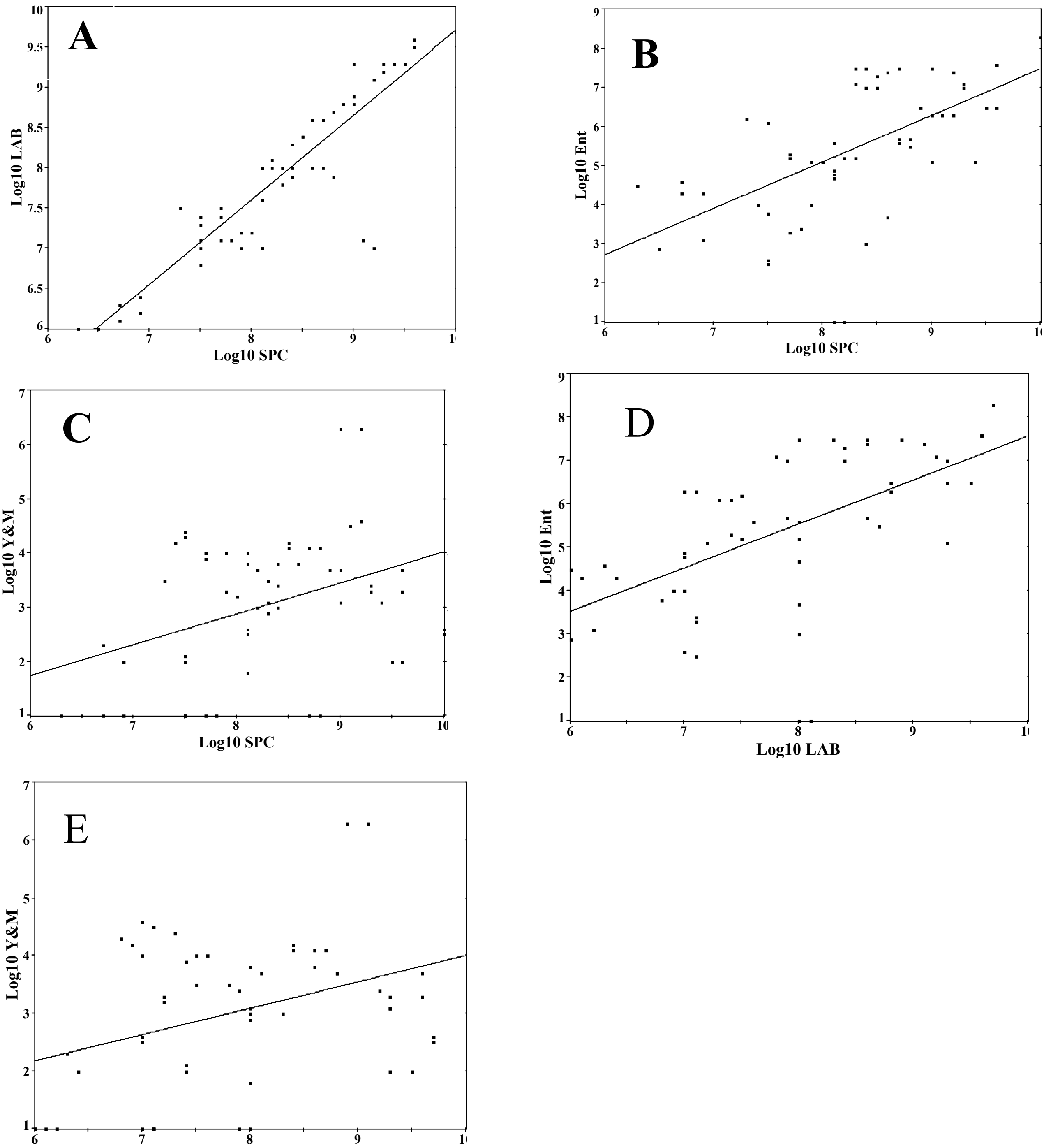


Figure 3. Correlation between standard plate count (SPC) and counts of lactic acid bacteria (LAB), enterobacteriaceae (Ent) and yeasts and molds (Y&M) of 30 soft white cheese samples collected from different governorates in Jordan (gaph A, B, and C, respectively), and correlation between LAB counts and counts of Ent and Y&M (graph D and E).

All the 30 cheese samples gave negative results on the confirmed biochemical tests of *Salmonella*, therefore, they were documented as zero in 25g cheese.

4.3 Physical and chemical survey

Physical and chemical measurements (pH, acidity, moisture, ash and salt (NaCl%) in brine and cheese) was measured for thirty soft white cheese samples collected from different Governorates.

4.3.1 pH and titratable acidity (lactic acid %) of cheese

Figure 4 shows pH and acidity of 30 soft white cheese samples collected from different Governorates in Jordan. The pH was ranged between 6.5 and 4.9 with an average of 6.0. The highest and the lowest pH samples were seen in Amman governorate. It was noticed that there was no significant difference ($p > 0.05$) in pH between the 30 cheese samples and between different Governorates.

Acidity (lactic acid %) ranged between 1.47 % and 0.24% with an average of 0.53 %.The highest acidity was seen in Al-Balqa Governorate, whereas the lowest in Amman. It was noticed that there was a significant difference in acidity ($p < 0.05$) between the 30 cheese samples, in addition there was no significant difference between different Governorates except between Amman and Al-Balqa from one hand and between Al-Balqa and Jerash from the other hand.

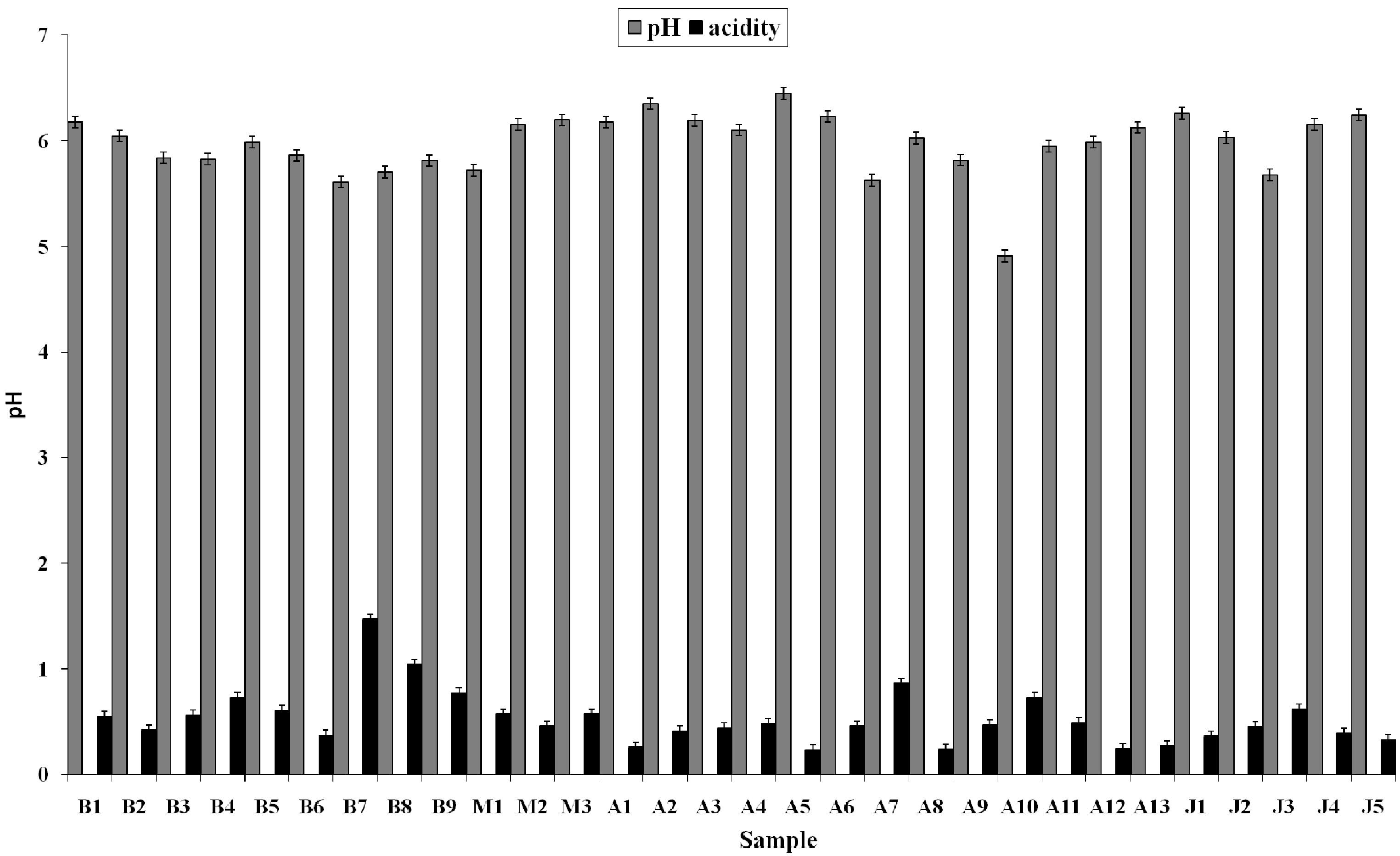


Figure 4. pH and acidity (% lactic acid) of 30 soft white cheese samples collected from four different governorates in Jordan. (B= Al-Balqa, M= Madaba, A= Amman, J= Jerash)

4.3.2 Moisture content %

Moisture ranged between 74.5 and 39.5 with an average of 56.5 (Figure 5). The highest moisture was sample was seen in Amman Governorate, whereas the lowest in Al-Balqa. It was observed that there was a significant difference in moisture content ($p < 0.0001$) among the 30 cheese samples, and there was a significant difference among Amman and each of Al-Balqa and Madaba from one hand and among Al-Balqa and Jerash from the other hand.

4.3.3 Salt (NaCl %) in brine and cheese

Salt (NaCl %) in brine and cheese ranged between 25.16 and 1.46 with an average of 12.63 for brine and 19.44 and 1.82 with an average of 9.37 for cheese (Figure 6). The highest and the lowest salt (NaCl%) in brine and cheese, were seen in samples of Madaba and Amman Governorates, respectively. It was observed that there was a significant difference in salt concentration of brine and cheese ($p < 0.0001$) among the 30 cheese samples, and there was a significant difference among all Governorates except between Al-Balqa and Madaba for salt in brine and cheese and between Amman and Jerash for salt in cheese.

4.3.4 Ash determination

Ash ranged between 3.3 and 17.3 with an average of 9.5 (Figure 7). It was observed that there were significant differences in ash ($p < 0.0001$) among the 30 cheese samples, and there were significant differences among all Governorates except between Al-Balqa and Madaba on one hand and between Amman and Jerash from the other hand.

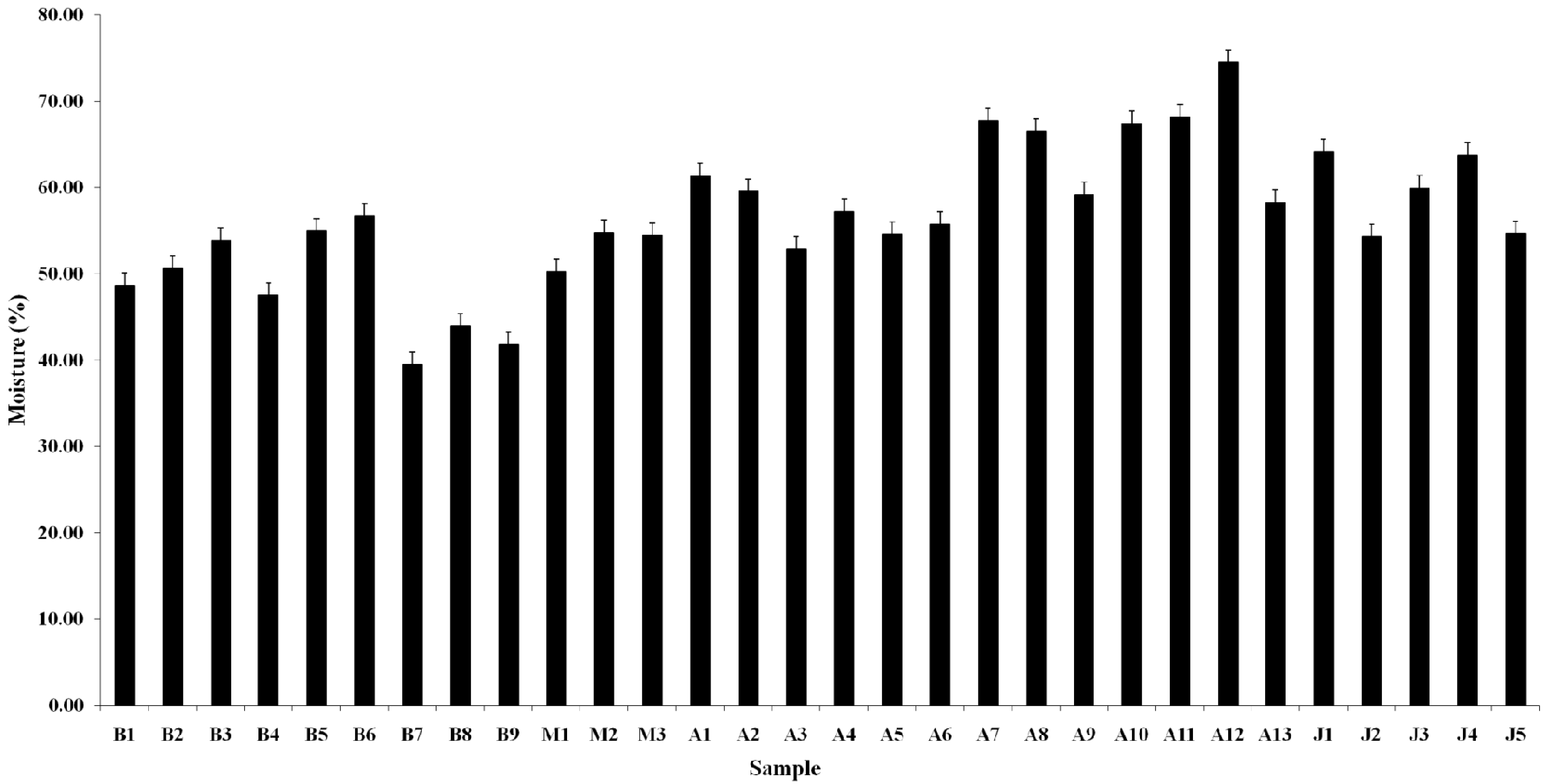


Figure 5. Moisture (%) of 30 soft white cheese samples collected from four different governorates in Jordan.
(B= Al-Balqa, M= Madaba, A= Amman, J= Jerash)

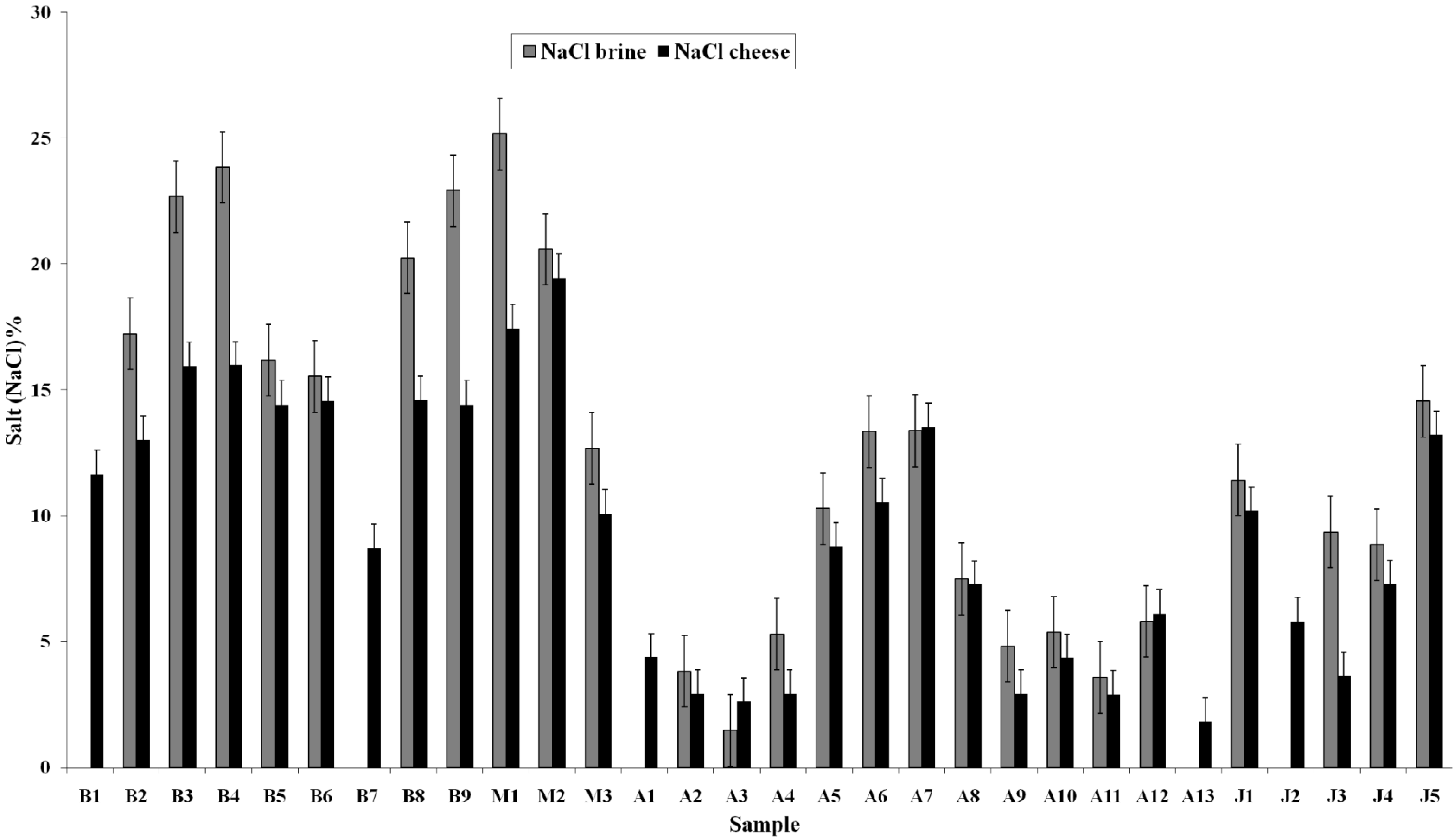


Figure 6. Salt (NaCl %) in brine and cheese of 30 soft white cheese samples collected from four different governorates in Jordan. (B= Al-Balqa, M= Madaba, A= Am

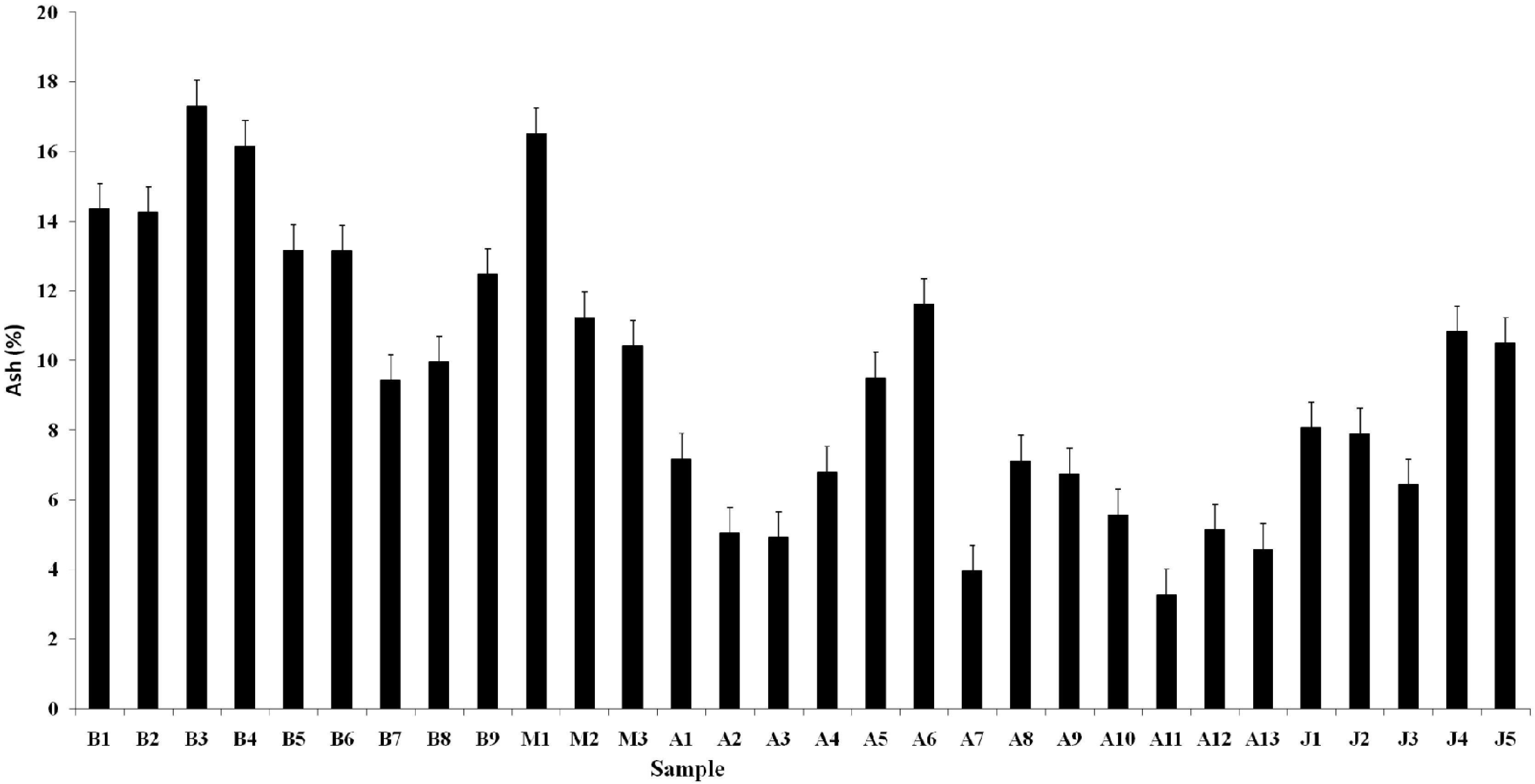


Figure 7. Ash (%) of 30 soft white cheese samples collected from four different governorates in Jordan
(B= Al-Balqa, M= Madaba, A= Amman, J= Jerash)

4.3.5 Phosphatase test

Fourteen cheese samples out of the 30 showed a positive phosphatase test. All cheese samples of Al-Balqa and Madaba Governorates gave positive result in phosphatase test, whereas, all samples collected from Jerash gave negative result in phosphatase. In Amman 4 out of 13 samples gave positive phosphatase test.

4.4 Correlation between physical, chemical, and microbiological measurements

Figure 8 and 9 show the correlation between SPC and LABC and pH, acidity, moisture, ash, and salt (NaCl %) in brine and cheese. There was a significant negative correlation between pH of the cheese and each of the SPC and LABC shown in graph A in the two figures (-0.40, -0.43, respectively). There was a significant positive correlation between acidity of cheese and each of SPC and LAB (graph B in the two Figures) (-0.38, -0.38 respectively). Graph C in the two figures shows an insignificant weak negative correlation between moisture and SPC and LAB (-0.12, -0.07). An insignificant weak positive correlation was noticed between ash and SPC and LAB (0.12, 0.03) (graph D), and salt (NaCl%) in brine (graph E) and cheese (graph F) (0.22, 0.16 for SPC, and 0.19, 0.11 for LAB).

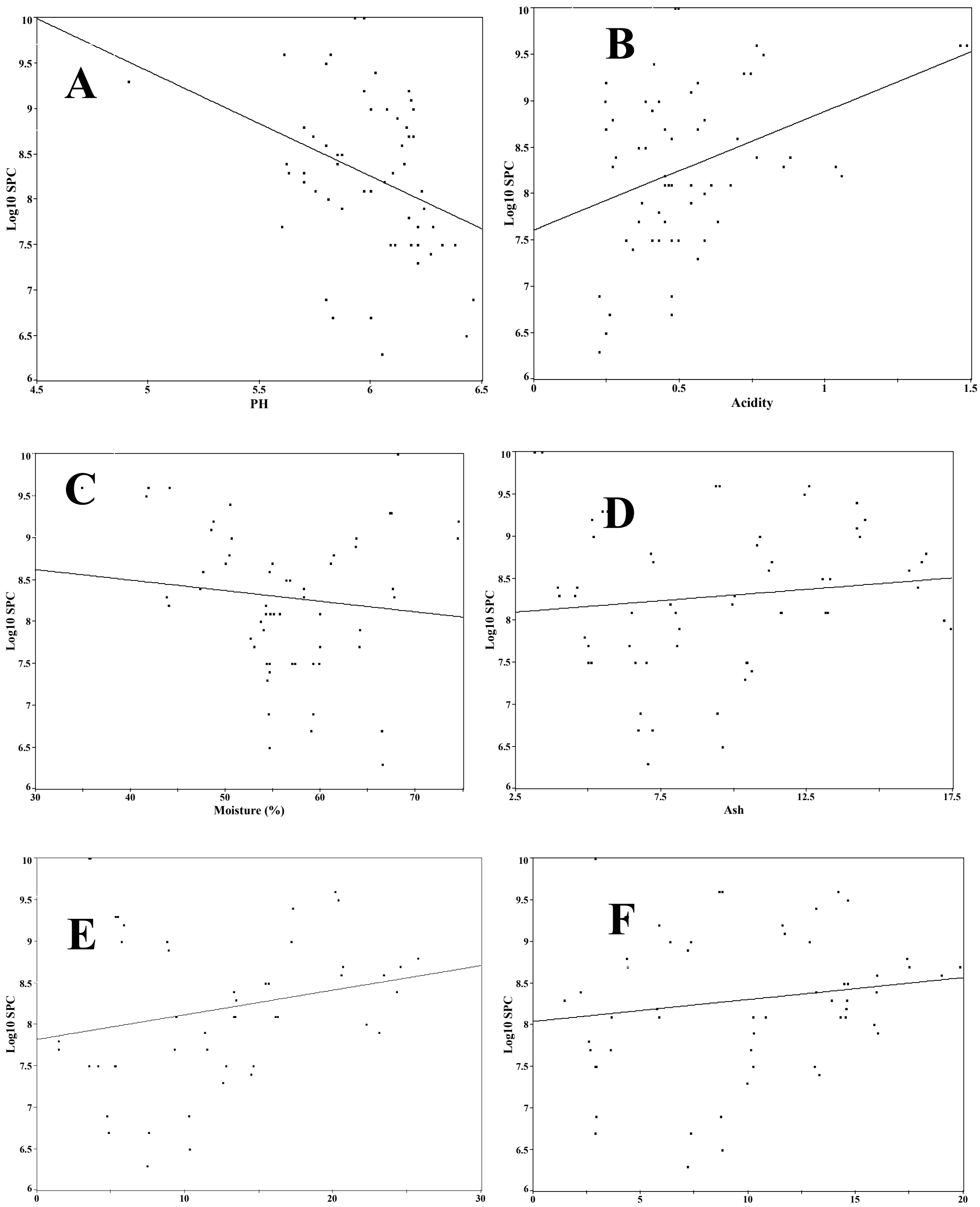


Figure 8. Correlation between standard plate count (SPC) and pH, acidity, moisture, ash, and salt (NaCl%) in brine and cheese (graph A, B, C, D, E, and F, respectively).

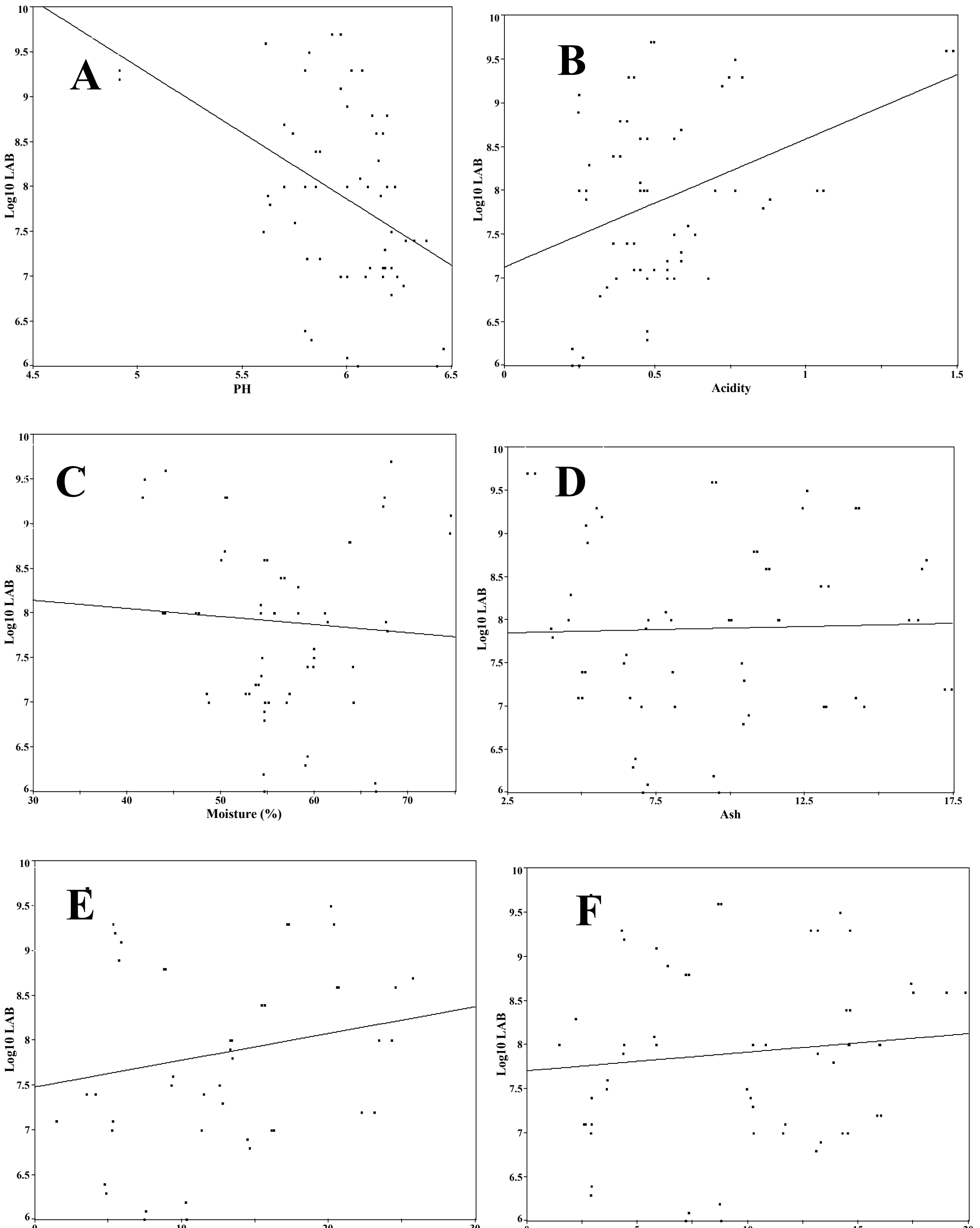


Figure 9. Correlation between lactic acid bacteria (LAB) and pH, acidity, moisture, ash, and salt (NaCl%) in brine and cheese (graph A, B, C, D, E, and F, respectively).

4.5 Probiotic cheese production

4.5.1 Culture selection

Bifidobacterium lactis FD BB-12® and *L. acidophilus* HOWARU NCFM® were chosen for the use in the production of soft white cheese as had been successfully used in preview studies related to production of probiotic dairy products (Tharmaraj and Shah, 2004 ; Sanders and Klaenhammer, 2001; Mustapha *et al*.,1997), familiar in milk (Østlie *et. al*.,2003), their successful experiments in cheese (O¨zer,2009; Fritzen-Freire *et al*., 2010), being isolated from human intestine (Sanders and Klaenhammer, 2001), presence of calcium improves their growth (Sanders and klaenhammer, 2001) , resistant to bile acids (Sanders and klaenhammer, 2001; Mustapha *et al*.,1997) and they have multiple confirmed health effects (Scheller and O'Sullivan 2011; Lahtinen, 2011; Shah, 2007; Shah, 2004; Sanders and klaenhammer, 2001; Mustapha *et al*.,1997).

4.5.2 Intermediate medium for probiotic addition to cheese

The probiotic counts of *Bifidobacterium lactis* and *L. acidophilus* on Nadec skim milk after activation for three successive times and more, fluctuated between 10^7 to 10^{11} CFU/ml. The pH of the previous cultured skim milk was between 4.1- 4.7 and 6.1- 6.6 for *L. acidophilus* and *B. lactis,* respectively. The morphology of cultures using methylene blue stain after 8, 24 and 48 h gave rounded ends densely packed, well-oriented rods for *L. acidophilus*, whereas short, curved, club-shaped, bifurcated Y-shaped, bifid form or pleomorphic rods that occur singly or in many-celled chains or clumps for *B.lactis.* This cultured milk was then available for cheese fortification.

4.5.3 Effect of salt (NaCl%) in brine on the acceptability of taste of soft white cheese

Half of the panelists preferred cheese kept in 6 % NaCl brine and 4 preferred the 8 %. (Table 2) Therefore, probiotic cheese in further testing was kept in 7% NaCl brine.

4.5.4 a preliminary experiment for dose determination of cultured skim milk

Before a preliminary cheese processing, the count of probiotic bacteria (*L. acidophilus* and *B. lactis)* was determined accurately after cultivation on the previous cultured skim milk. The count ranged between 2.9 x10^{11} CFU/ml and 3.9 x10^{10} CFU/ml for *L. acidophilus* and *B. lactis,* respectively. Whole fresh milk was coagulated to curd through cheese processing. After straining and most of the whey was expelled out, the curd weight was 5 Kg for the first curd portion used for *L. acidophilus* and 5.1 Kg for the second curd portion used for *B. lactis*. The 10^6 CFU/g was used to be the required probiotic dose to be achieved. Therefore, the two amounts of cultured skim milk contain either *L. acidophilus* or *B. lactis*, which required to achieve this dose, were calculated in proportion to the curd weight. The amounts of cultured skim milk increased to compensate some losses in whey by pressing and approximated to facilitate the calculations. Quantities measured accurately, diluted with sterilized distilled water, and distributed evenly by a sterilized pipette over the curd with mixing.

After cheese processing and packing in glass jars with brine, the jars left overnight. The counts of first cheese contained *L. acidophilus* was 4x10^5 CFU/g cheese with pH 6.5 and 7.0 for cheese and brine solution, respectively. Whereas, the counts of the second

Table.2 Results of the testing acceptability of taste of soft white cheese kept in brine with different salt concentration at 4 ºC/ 48 h.

Salt (NaCl %) in brine	0 %	2 %	4 %	6 %	8 %	10 %	12 %	14 %	16 %
No. of panelists	0	0	2	6	4	0	0	0	0

cheese contained *B. lactis* was 7.0 x 10^6 CFU/g cheese with pH 6.5 and 6.9 for cheese and brine solution, respectively. Based on these results we decided to multiply the calculated amounts of probiotic bacteria per g of curd with 5 (for the addition of probiotic to curd) and with 10 (for the addition of probiotic to milk) in the subsequent probiotic cheese production. The cultured skim milk was activated for another successive three times by inoculating on a new UHT skim milk media. Probiotic bacteria counts of the two cultured skim milk (*L. acidophilus* and *B. lactis)* were determined accurately before the probiotic cheese production.

4.5.5 Production of probiotic soft white cheese – testing of milk and cheese

Fat and the specific gravity of the fresh whole cow's milk used in the production of the cheese were 3 % and 1.031 respectively; the pH was 6.8.The LABC count of the control was ranged between 2.8 and 5.1 for zero and 21 d respectively. These counts had a spherical shape.

4.5.6 Production of soft white cheese in which probiotic bacteria were added to the milk or curd

a. Changes in probiotic bacteria count

Figure 10 shows changes in probiotic bacteria count in soft white cheese when kept at 4 ºC for 21 d. With the exception of *Bifidobacterium lactis* count, counts were >10^6 CFU/g directly after production of the cheese and remained at these levels after a week of storage.

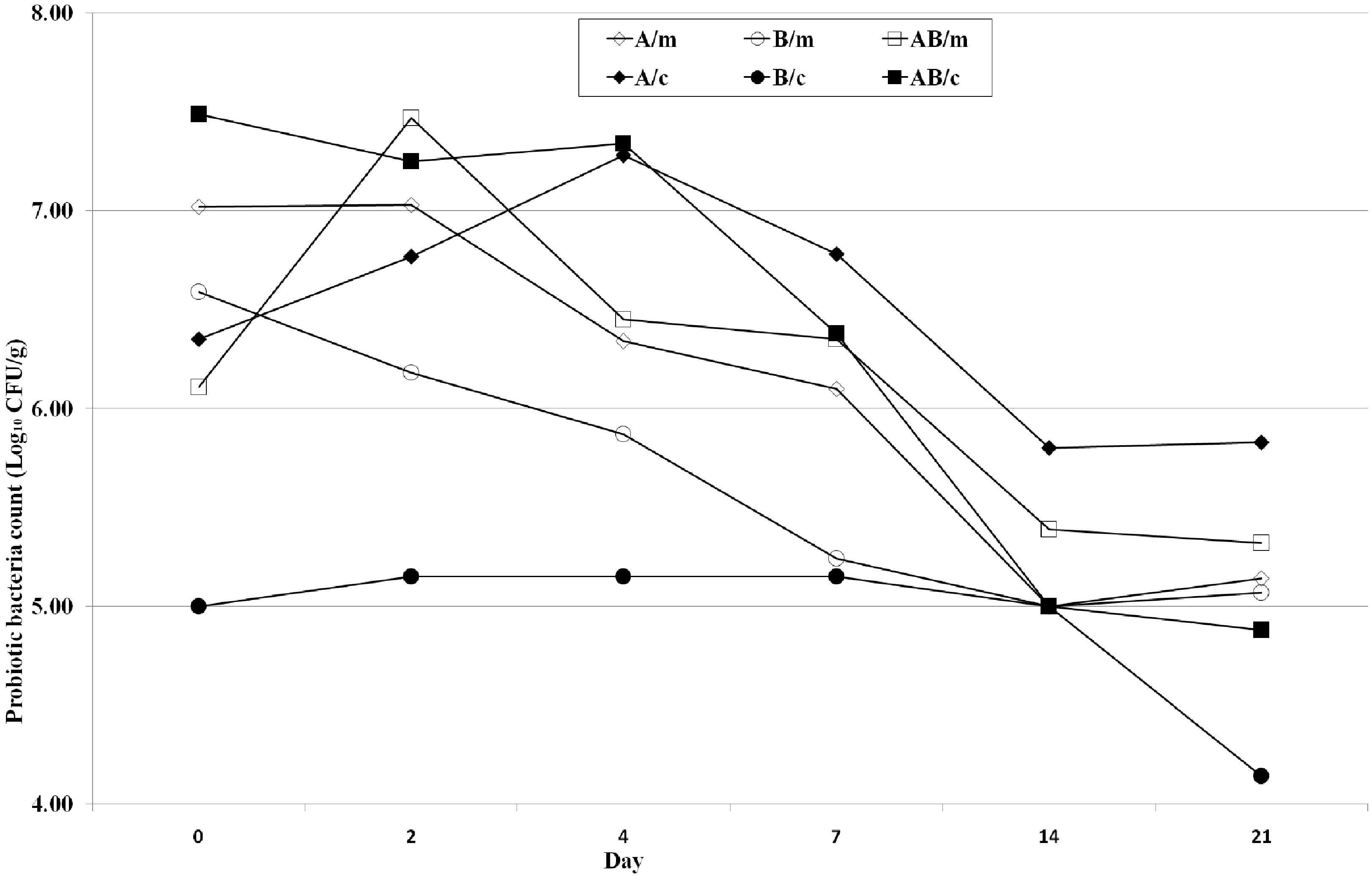


Figure 10. Development in the counts of probiotic bacteria used in the production of soft white cheese when added to the milk or the curd, during refrigerated storage (4 ºC).
(A= *L. acidophilus*, B= *Bifidobacterium lactis*, m= addition to the milk, c= addi

A drop in the counts was noticed during the refrigerated storage. After 2 wk counts remained ≥ 10^5 CFU/g in all cheeses. Generally, counts of *B. lactis* were lower than *L. acidophilus* counts.

There was a significant difference ($p<0.0001$) between the counts of each of the probiotic cheese types directly after production (Figure 10). At 2 d there was a significant difference ($p<0.0001$) among the counts of probiotic cheese types. The significant difference was among A/m and A/c , B/m and B/c but there was no significant difference among AB/m and AB/c. At 4 d, there was a significant difference ($p<0.0001$) among the counts of probiotic cheese types. The significant difference was among Am and A/c, B/m and B/c, and AB/m and AB/c. At 7 d, there was a significant difference ($p<0.001$) among the counts of probiotic cheese types. The significant difference was among A/m and A/c, but there was no significant difference among B/m and B/c, and AB/m and AB/c. At 14 d**,** there was no significant difference among the counts of probiotic cheese types. There was only a significant difference among Ac and all other types of probiotic cheese. At 21 d, there was a significant difference ($p<0.01$) among the counts of probiotic cheese types. The significant difference was among A/m and A/c, B/m and B/c, and AB/m and AB/c.

a. Changes in pH and salt (NaCl %) of brine and cheese

Figures 11-16 show changes in pH and salt (NaCl %) in the brine and cheese during storage at 4 ºC/ 21 d. Generally, pH of the brine and cheese remained almost constant throughout the experiment, with the averages 6.7 and 6.5 respectively.

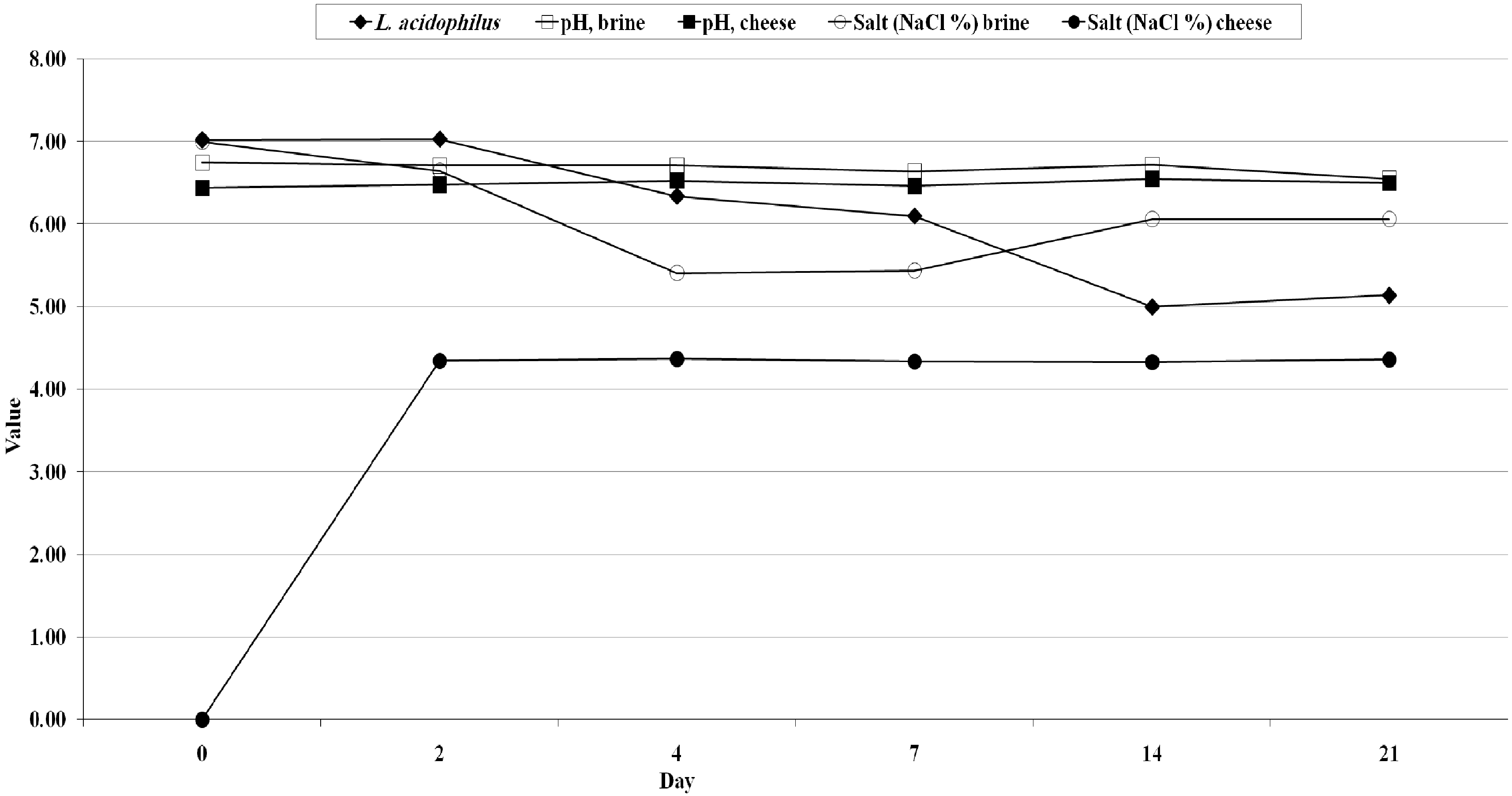


Figure 11. Changes in *Lactobacillus acidophilus* count, pH and salt (NaCl %) in brine and cheese during refrigerated storage (4 °C) of soft white cheese to which *L. acidophilus* was added to the milk.

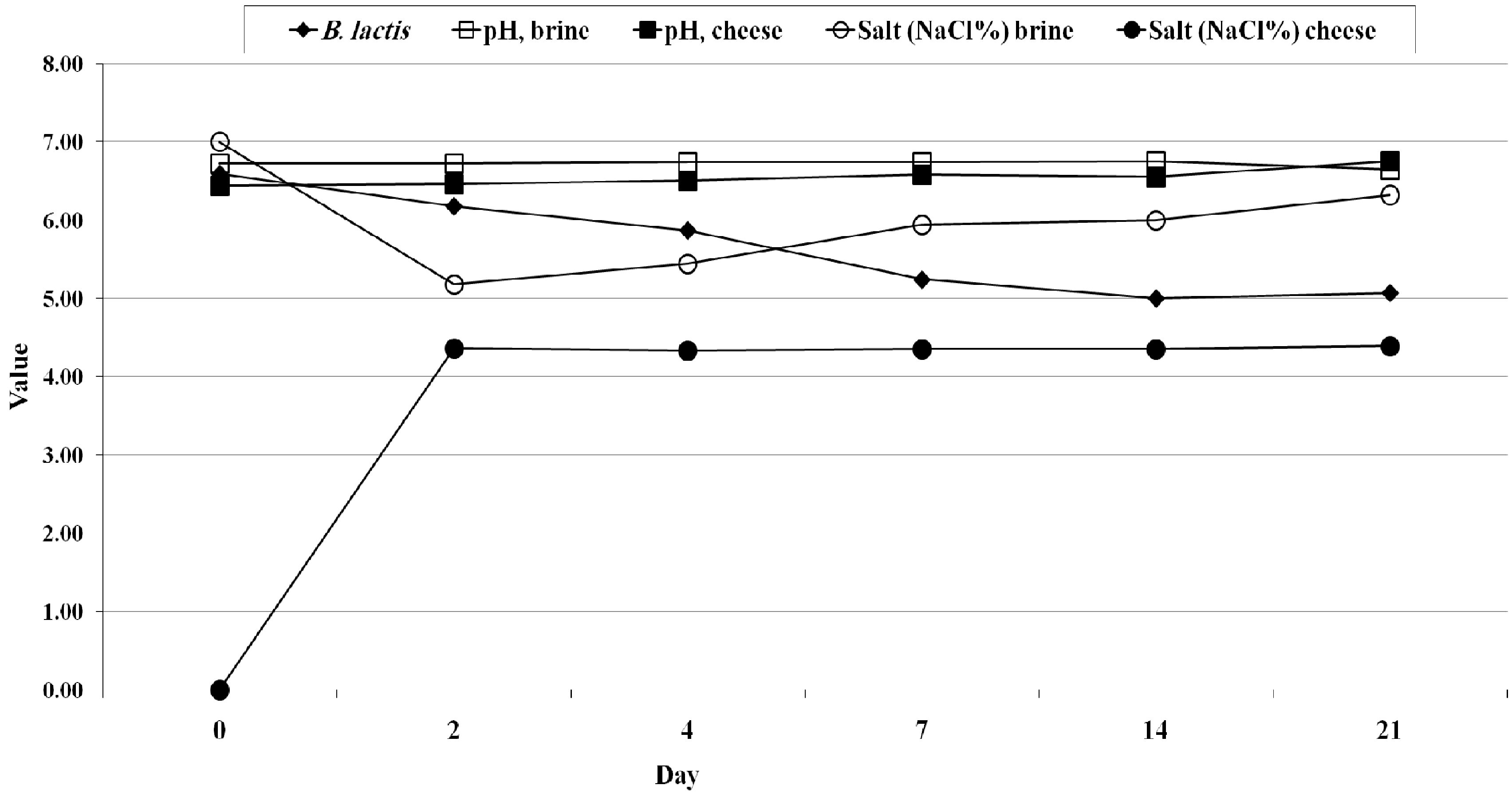


Figure 12. Changes in *Bifidobacterium Lactis* count, pH and salt (NaCl %) in brine and cheese during refrigerated storage (4 ºC) of soft white cheese to which *B. lactis* was added to the milk.

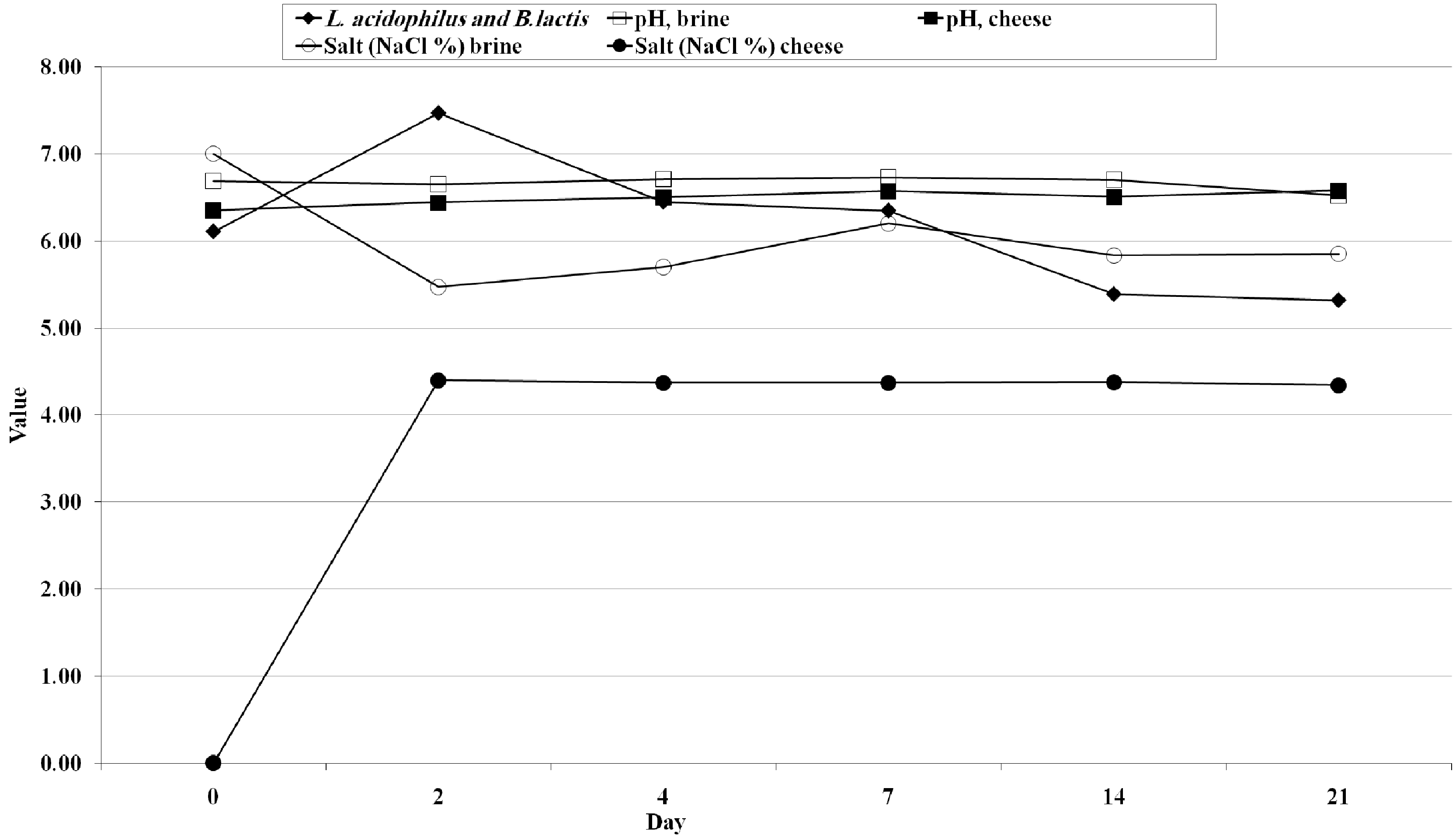


Figure 13. Changes in probiotic bacteria count, pH and salt (NaCl %) in brine and cheese during refrigerated storage (4°C) of soft white cheese to which both *Lactobacillus acidophilus* and *Bifidobacterium lactis* was added to the milk.

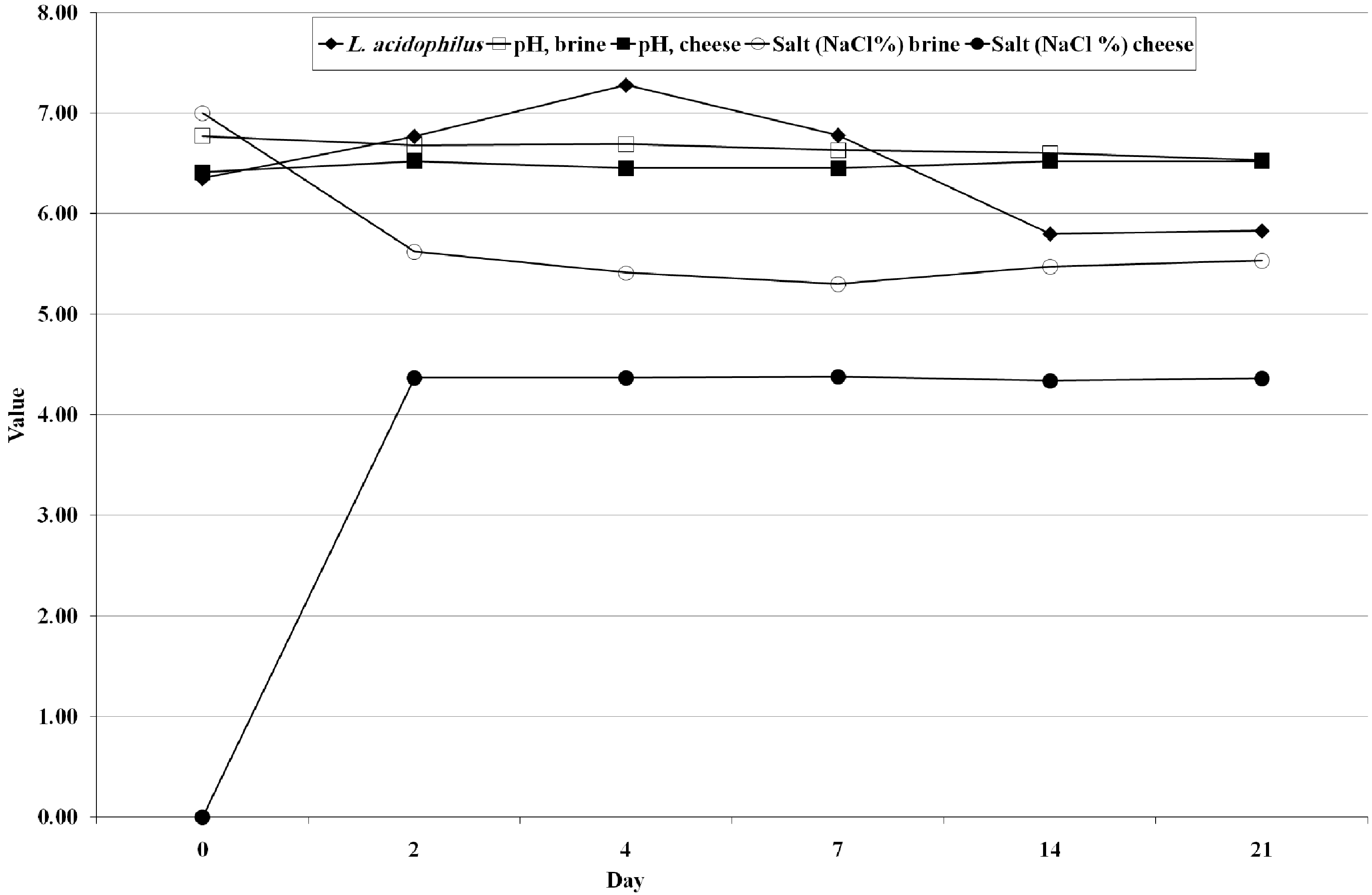


Figure 14. Changes in *Lactobacillus acidophilus* count, pH and salt (NaCl %) in brine and cheese during refrigerated storage (4 ºC) of soft white cheese to which *L. acidophilus* was added to the curd.

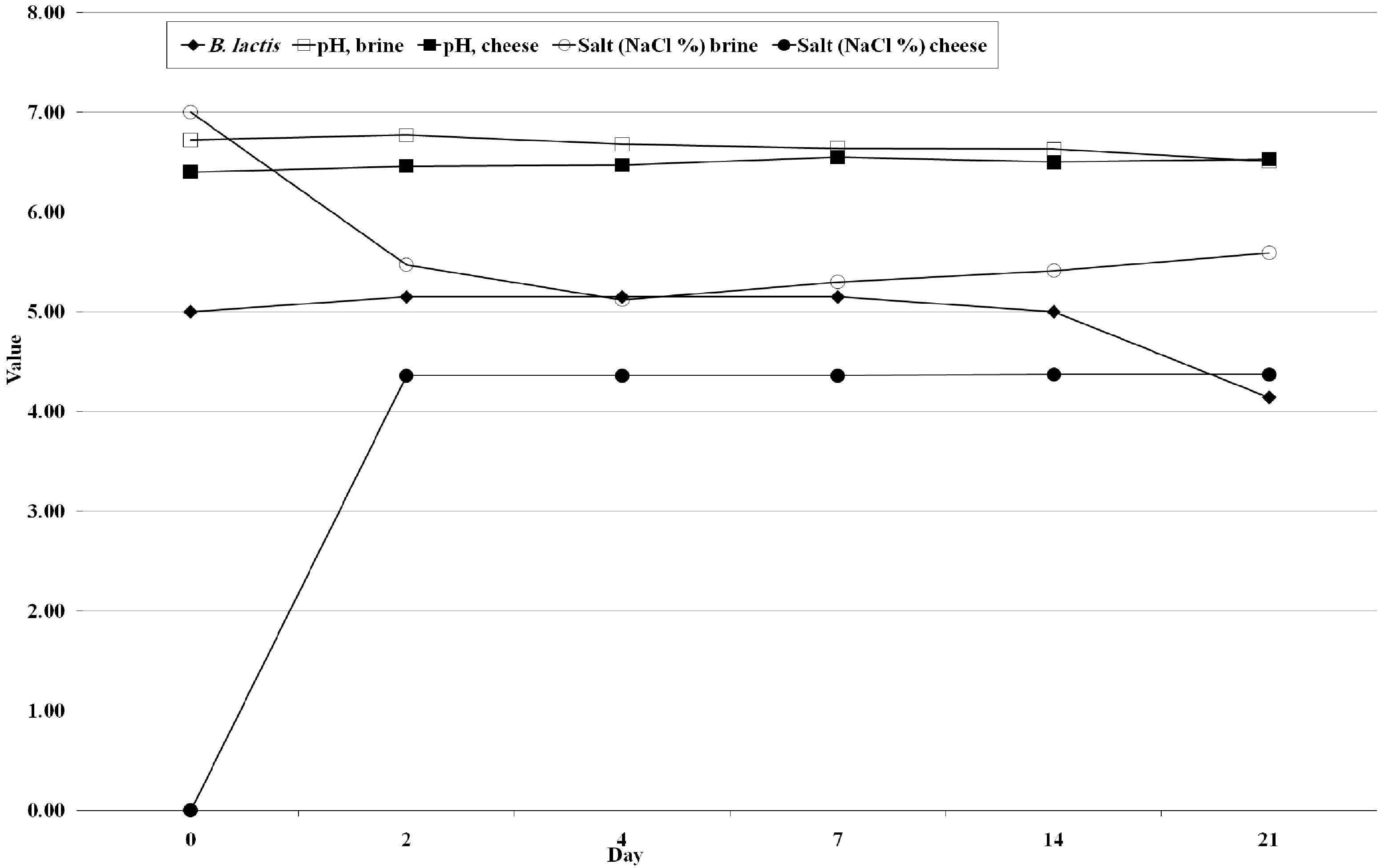


Figure 15. Changes in *Bifidobacterium lactis* count, pH and salt (NaCl %) in brine and cheese during refrigerated storage (4 °C) of soft white cheese to which *B. lactis* was added to the curd.

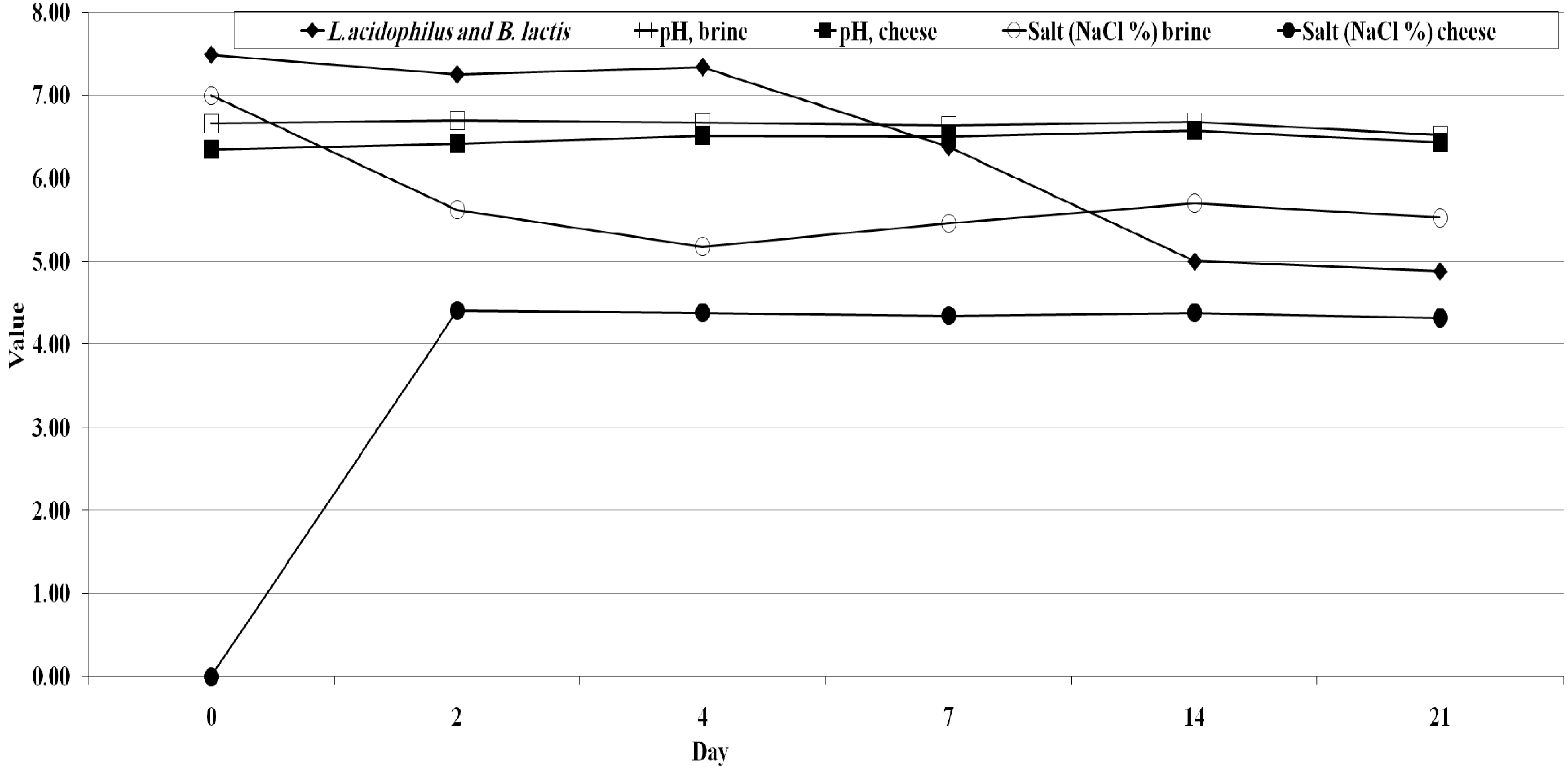


Figure 16. Changes in probiotic bacteria count, pH and salt (NaCl %) in brine and cheese during refrigerated storage (4 ºC) of soft white cheese in which *Lactobacillus acidophilus* and *Bifidobacterium lactis* were added to the curd.

A rise in salt (NaCl %) was noticed in the cheese at the second day of storage, and remained almost constant throughout the experiment in all cheeses. A drop was noticed in salt concentration in the brine at the second day followed by a slight rise near the end of the experiment.

4.5.7 Sensory evaluation of cheese

Summary of the results of the sensory evaluation of probiotic soft white cheese produced using *L. acidophilus* and *B. lactis* during storage for 21 d at 4 ºC are shown in table 3. With the exception of the cheese produced using a combination of *L. acidophilus* and *B. lactis* in the curd and *L. acidophilus* in the curd, no significant differences were noticed regarding appearance of the cheese. The only significant difference in smell was noticed between the cheese produced using a combination of *L. acidophilus* and *B. lactis* in the milk and cheese produced using a combination of *L. acidophilus* and *B. lactis* in the curd. A significant difference in taste was noticed between cheese produced using *B. lactis* in the milk and cheese produced using a combination of *L. acidophilus* and *B. lactis* in the curd, where cheese produced using *B. lactis* in the milk had the highest acceptability. Results of the other treatments were in between. Regarding consistency, control, cheese produced using *B. lactis* in the milk and curd, cheese produced using a combination of *L. acidophilus* and *B. lactis* in the curd had the highest scores with no significant difference. Cheese produced using *L. acidophilus* and *B. lactis* in the curd was significantly the least acceptable

Table 3. Results of the sensory evaluation of testing probiotic soft white cheese samples produced using *Lactobacillus acidophilus*, *Bifidobacterium lactis* and a combination of the two bacteria during storage between 0-21 d at 4ºC.

Cheese	Sensory attribute					
	Appearance	Smell	Taste	Consistency	Overall acceptability	Average
Control	7.8^{a} **	7.4^{ab}	7.4^{abc}	7.6^{a}	7.4^{abc}	7.5
A/m*	7.5^{a}	7.5^{ab}	7.4^{abc}	7.4^{ab}	7.4^{bc}	7.4
A/c	6.9^{b}	7.2^{ab}	7.0^{cd}	7.0^{bc}	7.1^{dc}	7.0
B/m	7.8^{a}	7.5^{ab}	7.7^{a}	7.7^{a}	7.8^{a}	7.7
B/c	7.4^{a}	7.2^{ab}	7.2^{bcd}	7.6^{a}	7.4^{bc}	7.4
AB/m	7.8^{a}	7.6^{a}	7.5^{ab}	7.7^{a}	7.6^{ab}	7.6
AB/c	6.9^{b}	7.1^{b}	6.8^{d}	6.9^{c}	6.8^{d}	6.9

*A/m= *L. acidophilus* addition to the milk, A/c= *L. acidophilus* addition to the curd B/m= *B. lactis* addition to the milk, B/c= *B. lactis* addition to the curd, AB/m=*L. acidophilus and B. lactis* addition to the milk, AB/c= *L. acidophilus and B. lactis* addition to the curd.

** Scores that had the same letters are not significant, but there was a significant difference found between those that had different letters.

regarding consistency. Accordingly and as could be seen from the overall testing and averages of the scoring, cheese produced using *B. lactis* in the milk was the most acceptable, followed by cheese produced using a combination of *L. acidophilus* and *B. lactis* in the milk and control. Cheese produced using a combination of *L. acidophilus* and *B. lactis* in the curd was the least acceptable but was not rejected by the panelists.

5. DISCUSSION

5.1 Microbiological quality and safety of soft white cheese

Soft white cheese is popular in many countries, including Jordan. Many research works have been carried out on chemical, microbiological, production and sensory aspects of this cheese in Jordan (Abu-Alruz, *et al.,*2009, Haddadin, 2005, Tubesha, 2002, Abu-Alruz, 1999, Saleh, 1998, Yamani *et al.,*1998, Haddadin, and Shahin, 1995, Herzallah, 1994, Humeid *et al*., 1990, Abu-Ghoush, 1992 and Humeid and Tukan, 1986) and many other authors fom various countries. However, it was a surprise to find out that no study has been carried out on the microbiological quality of this type of cheese in Jordan. Soft white cheese is also popular in neighboring countries, where a good number of studies had been carried out on quality and production aspects, (El-Sayed *et al*., 2011; El Owni and Hamid, 2008; Mirazaei *et al*., 2008; Hamid and El- Owni, 2007; Temelli *et al*., 2006, Sengul, 2006; Hayaloglu *et al*., 2002).

The lack of standardized method for production and keeping of soft white cheese in Jordan, as can be concluded from the questionnaire, has resulted in the significant variations noticed in the different quality characteristics of the cheese samples.

The high microbial content of the cheese samples, as seen in the SPC and LABC, reflects the general hygiene used conditions during production and storage, lack of refrigeration, and absence of steps, such as heat treatment to eliminate microorganisms in the most of the tested samples. It can be postulated that the bacterial growth has mostly taken place in the milk and during the steps of production before placing the cheese pieces

in brine. Fortunately, the predominant microorganisms were LABC which forms the major part of the microbial flora of fresh dairy products and which have no negative effect on the safety of the products.

Enterobacteriaceae counts were generally high and were in most samples unacceptable. Presence of Enterobacteriaceae in food samples generally indicates direct or indirect fecal contamination of the milk or the product during processing, and thus the possibility of having pathogenic bacteria, viruses, or protozoa of fecal origin in the food.

The relatively low counts of yeasts have no effect on the quality or the safety of the cheese. Growth of yeasts in this type of cheese is not encouraged by the presence of LAB and having in this cheese intrinsic conditions, which encourage bacterial rather than fungal growth.

Consumption of soft white cheese has been associated with food borne illnesses in Jordan and neighboring countries. Some of these illnesses are very serious, such as Malta fever caused by *Brucella* spp. Although *S. aureus* count was low in the samples and *Salmonella* was absent, soft white cheese should be considered as a potential safety hazard. This is because of the absence of pasteurization in about 50 % of the samples, and the poor hygiene conditions during production (as indicated by the high SPC and LABC, and the relatively high numbers of Enterobacteriaceae).

Soft cheeses have higher moisture content when compared to hard cheese and have lower shelf life due to microbial spoilage. Most soft, unripened cheeses are microbiologically unstable due

to metabolic activity of bacteria, yeast or mould contaminants (Tamime and Kirkegaard, 1991; Farkye and Vedamuthus, 2002).

It has been reported that the means of results of the microbiological analyses of the brined Turkish soft white cheese stored at 4 ºC were 6.1, 3.1, 3.9, 2.9 $\log_{10}$ for total aerobic mesophoilic bacteria, Enterobacteriaceae, Staphylococci, and yeast and mold count, respectively (Temelli *et al*., 2006). Sayed *et al*., (2011) found that Domiati and Tallaga cheese variety samples showed the higher aerobic colony bacterial counts (ACC) and the worst microbiological quality, as reached ~10^{11} to 10^{12}CFU/g as a maximum count with an average ~10^{7} CFU/g. Meanwhile, Kariesh cheese variety, acid coagulation, showed higher molds and yeast count and the worst mycological quality as it reached $8x10^{8}$ CFU/g with an average of $3x10^{4}$ CFU/g. *Salmonella* spp, has been only isolated from Domiati and Tallaga cheese varieties in 3 and 7 % of the samples, respectively. The average total bacterial count for fresh white cheese samples collected from Tammoun and Tell villages in Palestine was $\log_{10}$ 7.7 CFU/g (Fahed, 2003). The present study demonstrated that all samples of fresh white cheese were negative for *Salmonella*. About 36% of samples of fresh soft white cheese had high levels of contamination by *S. aureus*.

Recent studies showed similar microbiological characteristics in Sudanese white cheese (Gibna Bayda) (El Owni and Hamid, 2008), where at 0 d the total bacterial count (TBC), *S. aureus* and yeast and mold count were 7.5, 3.0, 2.7 $\log_{10}$ CFU/ml of cheese. Total bacterial count (TBC), coliforms, *E. coli*, *S. aureus* and psychrotrophic bacterial counts significantly ($p<0.05$) decreased during storage, while yeasts and molds increased as storage time progressed. A Study by Hamid and El Owni, (2007) on soft white cheese

showed that the microflora was dominated by lactic acid bacteria (LAB) with evident mold and yeast growth. The previous study found that the average microbiological properties were 9.7 log_{10} CFU/ml for total bacterial count; 3.3 log_{10} MPN/ml coliforms; 0.70 log_{10} MPN/ml *E. coli;* 1.7 log_{10} CFU/ml *S. aureus;* 5.1 log_{10} CFU /ml lactic acid bacteria; 3.7 log_{10} CFU /ml streptococci and 4.5 log_{10} CFU /ml for yeast and mold counts. However, *Salmonella* spp. and *Clostridium* spp. were not detected in all the collected cheese samples.

The average total bacterial count in Serbian soft white cheese (Stojiljkovic, 2009) at 1 d brining was 7.3 log_{10}. Pešić-mikulec and Jovanović,(2005) estimated the averages of log_{10} of aerobic mesophilic flora in fresh white cheese 9.3. Lactic acid bacteria were the major microbial group, reaching count similar to the total aerobic mesophilic flora at all sampling points of this study log_{10} of (9.2), where as the counts of Enterobacteriaceae and mold and yeast counts were 4.2 and 5.6, respectively.

The mean values of coliforms bacteria, *S. aureus*, molds and yeasts of Iranian white brined cheese (Mirzaei *et al*., 2008) were 4.1×10^2, 4.5×10^3, 4.5×10^3 and 9.2×10^4 CFU/g , respectively. Also, the number of coliforms, *S. aureus,* and molds in 10.1, 10.1 and 34.2% of the samples were higher than the limits allowed by the national standard for Iranian industrial white ripened cheese. Furthermore, *E. coli*, faecal coliforms, and positive coagulase *S. aureus* were isolated from 58.4, 62.9, and 18% of the samples, respectively, but also no *Salmonella* was found in their examined samples. Chemical parameters such as pH was 4.7, titratable acidity 1.4, dry matter 41.6%, NaCl 4.3 % and ash 5.9% were also determined.

According to the Jordanian standard (JS 246: 2006), salt (NaCl %) should not exceed 10% wt. /wt. in the final product, calculated as wet weight, whereas acidity (lactic acid %) should not exceed 1.9%. Table 4 shows comparisons between Jordanian (JS 246: 2006), Palestinian (Fahed, 2003**),** and Egyptian (Sayed *et al.,* 2011) standards for soft white cheese (CFU/g).

There is a report demonstrated that some pathogenic microorganisms, including *L. monocytogens,* and *S. aureus* in found soft cheese (Ryser, 1998). *S. aureus,* were isolated from different Egyptian varieties of white soft cheese in rates of 25.7 %, respectively (Sayed *et al*., 2011). Therefore, the presences of these pathogens in 26% of the total white soft cheese samples were not accepted according to the Egyptian standard (ES) 1008-2000. In a study of brined Turkish cheese, it was shown that *Staphylococcus* had low numbers (ranged from 1.3 to 1.7 $\log_{10}$ CFU/g) of S. *aureus*, were *Salmonella* spp. and *C. perfringens* not present from the cheese samples (Turantas *et al*., 1989).

A study by Al-As'ad (2000) showed that the positive rate among *Brucella* samples in Syrian soft white cheese were 3.8% from a total number of 394 samples tested. The study added that the presence of *Brucella* in soft white cheese is proportional to the concentration of salt, since the presence of *Brucella* would be higher % in unsalted than salted cheese.

Based on the findings of this study, there is a need for developments urgently needed to improve the quality and ensure the safety of the product. Baledi or Jibnah Khadra mountain soft cheese produced in Lebanon did not preserve well and many cases of brucellosis

Table 4. A Comparison between Jordanian, Egyptian, and Palestinian soft white cheese standards (CFU/g).

Microbiological item	Jordanian JS 246: 2006	Egyptian ES 1008-2000	Palestinian PS (White cheese and dairy products) 1999
Mold count	--	not exceed 100	100
Yeast count	--	not exceed 400	2000
Coliform	--	not exceed 10	--
E. coli	free	free	--
L. monocytogens	free	free	--
S.aureus	free	free	< 100
Salmonella	free	free	free

--: not found in the standard.

infections have been associated with their consumption (Tannous, 1991). It is coustomary in Jordann in the previous to drink raw milk and eat fresh cheese; however, this habit has been found to be an important epidemiological factor in contracting brucellosis (El-Daher *et al*., 1990; Abu Shaqra, 2000).

In this regard the following is proposed: Use of clean milk from microbiological point of view (free of pathogens and has a low microbial load). Also, pasteurization of milk should be mandatory step in the production (as required by the Jordanian standard for soft cheese (JS 246: 2006)**)**, avoid cross contamination, application of good manufacturing practice (GMP) during production, and maintenance of the cooling chain from milking till consumption of the cheese. Testing of soft white cheese for phosphatase should be a part of the governmental quality control testing of the product. To have an application of these conditions, calls for action from private and governmental interested parties is required.

5.2 Production of Probiotic Soft White Cheese

The production of probiotic dairy products based on fermented milk has an established tradition, which is reflected in the global availability of commercial probiotic cultured milk; However, production of probiotic cheese is still in its early stages. The first attempts to produce probiotic cheese may be the using of dressing fermented by *B. infantis* in the production of cottage cheese (Blanchette *et al*., 1995; Blanchette *et al*., 1996). Several types of cheeses were tested as a potential vehicles for probiotic strains of *Lactobacillus* and *Bifidobacterium*. Most of the cheeses tested succeeded in maintaining the

viability of these microorganisms, as well as in achieving appropriate technological and sensorial properties of the final product. These have included Cheddar (Dinakar and Mistry, 1994; Gardiner *et al.*, 1998; Mc Brearty *et al*., 2001), Gouda (Gomes *et al*., 1998b); Crescenza (Gobbetti *et al*., 1998) and fresh cheeses (Roy *et al*., 1997; Vinderola *et al*., 2000; Buriti *et al*., 2005a, 2005b).

In Finland, *L. acidophilus* and *Bifidobacterium* spp. were used to produce a low-fat cheese (Ryhänen *et al*., 2001). These organisms apparently produced bioactive peptides with anti-hypertensive properties during maturation of the cheese, thus adding to the nutritional and probiotic values of the product. Beside the health promoting properties probiotic bacteria in cheese may also serve technological useful functions. Some strains of *bifidobacteria* produce anti-microbial compounds, which reduce the levels of *Pseudomonas* in cottage cheese (O'Riordan and Fitzgerald, 1998). There are commercial probiotic cultures of *L. rhamnosus* and *Propionibacterium freudenreichii* subsp. *Shermanii* with anti-clostridial effects and activity against cheese contaminating yeasts and molds (Hansen, 1997).

Besides selecting the suitable probiotic bacteria, two major difficulties are facing the production of probiotic cheese, namely determination of the right step of cheese production at which to add the probiotic bacteria (in the right concentration) and the long shelf life of ripened cheese. Because probiotic bacteria are generally fragile, their viability could be greatly affected by these production variables. So, the production of cheeses, in ripened types with probiotic bacteria presents unique challenges because of the need for co-survival of these bacteria with the conventional lactic acid bacteria, molds, or yeasts that are used

for cheese making. These microorganisms may be antagonistic, competitive, or symbiotic towards each other. Traditionally, no starter culture is used in the production of the soft white cheese, so interaction of probiotic bacteria used in the production, especially if the cheese is produced under hygienic conditions would be minimal.

To the best of our knowledge, this is the first time in Jordan to produce a probiotic product based on traditional dairy food. In this study, it was possible to produce a probiotic cheese using *L. acidophilus and B. lactis.* Both of these bacteria are established as a probiotic culture. All soft white cheese produced in this study were acceptable from sensoric point of view. Probiotic bacteria used in this study could not affect the sensory quality, because these bacteria have limited proteolytic and lipolytic activity (Lee and Salminen, 2009). Furthermore, the time between the introduction of the bacteria and covering the cheese pieces by brine is short, and no growth of the probiotic bacteria in the cheese when kept in the brine under refrigeration is expected, because these bacteria are not psychrotrophic in nature, and because salt has an inhibitory effect on their growth. Keeping the traditional soft white cheese in brine with 7 % salt (NaCl) concentration proved to be most suitable, since at this concentration the taste of the cheese was not negatively affected.

Various cheese varieties have been successfully used as carriers of probiotic microorganisms in different countries. Studies have demonstrated that Turkish white brined (Kasimoglu *et al*., 2004), feta-type (Psomas *et al*., 2001), cheddar (Gardiner *et al*., 1999), edam (Tungjaroenchai *et al*., 2004), emmental (Weinrichter *et al*., 2004), domiati (Mehanna *et al*., 2002), soft (El-Kholy *et al*., 2003), and cheese-based dips compare favorably with yoghurt as delivery systems for viable probiotic micro-organisms

(Tharmaraj and Shah, 2004). However, commercial production of probiotic cheese in the international dairy market, especially when compared with the probiotic cultured milk is still of low scale.

Inoculation of probiotics in milk before renneting resulted in almost half the cell losses in whey (Fortin *et al*., 2011) compared with addition just before cheddaring. The lower percentage of probiotics in curds for some strains could be due to better growth of the cultures in whey rather than in the curds during cooking, cheddaring and salting. A lower percentage of *B. longum* 15708 was noted in curds as compared with *B. lactis* BB-12. Manufacturers are obviously interested in the % of recovery in curds of the probiotics inoculated.

Songisepp *et al*. (2004) found that addition of lactobacilli into milk during production of based on the Estonian open-texture, smear-ripened, semisoft cheese "Pikantne" to be the preferred method of incorporation when compared with inoculation into drained curds. The first explanation is the lower viable counts of *Bifidobacteria* in the curds at the end of production are due to a greater loss in viability following inoculation when done at the cheddaring stage. The second reason is the very high losses of the probiotic bacteria in whey. Although the quantity of whey generated during cheddaring is much lower than that obtained after the first cutting, the population of probiotics in the cheddaring whey was ten times higher than in the whey obtained after the first cutting when probiotics were added to milk. Evidently, cells were not as well entrapped in the curd mass at cheddaring than at renneting.

Use of probiotics in trial pecorino ovine cheeses did not adversely affect preference or acceptability; in fact, panelists scored probiotic cheeses higher in preference over traditional cheese, even though not significantly. (albenzio *et al*., 2010). De Souza *et al*. (2008) stated that addition of *L. acidophilus*, either solely or in co-culture with a thermophilic starter culture, resulted in good acceptance of Minas fresh cheese (a typical Brazilian fresh cheese), improving sensory performance of the product during storage for up to 14 d.

In our experiment the bacteria were still viable to the end of the experiment. Most of the cheese treatments still have probiotic numbers above10^6 CFU/g until one wk. Numbers at 2 wk were less than 10^6 CFU/g. To overcome this we recommend to increase the inocula of each bacterium doubling the added amount of cultured skim that should be added to a new project for processing Jordanian probiotic cheese.

We regard it is better to add probiotic bacteria to the milk rather than to the curd, because addition to the curd did not result in having higher numbers of probiotic bacteria in the product. Furthermore, the direct addition to the milk is easier (saves time and effort), more hygienic and ensures better distribution of the bacteria in cheese.

It was reported that a maximum 3.5% (wt/wt) salt in goat cheese provided counts of *B. lactis* and *L. acidophilus* high enough for a probiotic effect (Gomes and Malcata, 1998a). It may be possible to produce cheese containing probiotic strains at levels higher than threshold for a probiotic effect (10^6 CFU/g) without impairing the quality of final product.

Gomes *et al*. (1995) proposed that a relatively large inocula, 3.5- 7.0% (corresponding to average levels of 2.0x10^9 CFU/g and (3- 4) x 10^9CFU/g, respectively), of probiotic strains were required to achieve the proper acidification rates in Gouda cheese. In another study, Buriti, *et al*., (2007) observed the viability of *L. acidophilus* La-5 and *B. animalis* Bb-12 added to Minas fresh cheese. Both probiotic cultures were present in high levels throughout storage, above 1x10^6 CFU/g, and resulted in cheeses with better texture comparable to the traditional ones and with favourable sensorial features.

The viability of *Bifidobacteria* differed in 14-d old crescenza cheeses (a soft ripened cows milk cheese) of *B. bifidum* , *B. longum,* and *B. infantis* were $\log_{10}$ 8.1,7.1,and 5.2 CFU/g, respectively (Gobbetti *et al*, 1998). The multispecies mixtures only reached $\log_{10}$ 5.0 CFU/g of cheese. While the counts of probiotic bacteria decreased approximately 3 log in the control cheese in which probiotics were used as free cells, the decrease was more limited in the cheeses containing microencapsulated cells (approximately 1 log) (O¨zer *et al*, 2009).

Probiotic C2 (without addition of lactic acid) and C3 (with addition of lactic acid) showed a count of viable cells for *Bifidobacterium* Bb-12, of 7.7 and 7.7 log CFU/g on day 1, respectively; and 7.73 and 7.93 log CFU/g, on day 28; respectively (Fritzen-Freire, 2010). Moreover, the count of viable probiotic cells obtained was above the recommended levels for a probiotic food throughout the whole storage period (Boylston *et al*., 2004; Shah, 2007), i.e., equal to or greater than 6 log CFU/g of the product (Gomes and Malcata, 1998a).

6. CONLUSIONS AND RECOMMENDATIONS

From the results of this study, the following could be concluded and recommended.

6.1 Conclusions

1. Lack of standardized method for production and keeping of soft white cheese in Jordan had resulted in the significant variations noticed in the different quality characteristics of the cheese samples.
2. The high microbial content of the soft white cheese samples reflects the poor general hygiene conditions during production and storage, lack of refrigeration and absence of steps such as heat treatment to eliminate microorganisms.
3. Bacterial growth in commercial soft white cheese takes place mostly in the milk and during the steps of production before placing the cheese pieces in brine.
4. The predominant microorganisms of soft white cheese are LABC, which have no negative effect on the safety of the products.
5. Enterobacteriaceae counts in soft white cheese indicate direct or indirect fecal contamination of the milk or the product during processing, and thus the possibility of contamination with pathogenic bacteria, viruses, or protozoa of fecal origin.
6. Yeast growth in soft white cheese is not encouraged by the presence of LAB and the intrinsic conditions of the cheese, and this leads to the fact that the microbial flora of this cheese is bacterial rather than fungal.
7. Lack of pasteurization of the milk used in cheese production in about 50 % of the samples makes the cheese produced a potential safety hazard.

8. Keeping the traditional soft white cheese in a brine with 7 % salt (NaCl) concentration proved to be most suitable, since at this concentration the taste of the cheese was not negatively affected.
9. This is the first time in Jordan to produce a probiotic product based on traditional dairy food using *L. acidophilus and B. lactis.*
10. All probiotic soft white cheeses produced in this study were acceptable from sensoric point of view. Cheese produced using *B. lactis* in the milk was the most acceptable, followed by cheese produced using *L. acidophilus* and *B. lactis* in the milk and control. Cheese produced using *L. acidophilus* and *B. lactis* in the curd was the least acceptable but was not rejected by the panelists.

11. It has been proved that probiotic bacteria were still viable in the cheese to the end of the experiment. Most of the cheese treatments used still have probiotic numbers above10^6 CFU/g until one wk.

6.2 Recommendations

1. Cheese producers should use clean milk (free from pathogens and has low microbial load) in the production.
2. Cheese producers should avoid cross contamination, application of good manufacturing practice (GMP) during production and maintenance of the cooling chain from milking till consumption of the cheese.
3. Pasteurization of milk should be a mandatory step in the production of soft white cheese.
4. Testing of soft white cheese for phosphatase should be applied as part of qulity control by manufacturing and governmental authorities.
5. Add probiotic bacteria to the milk rather than to the curd, because addition to the curd will not result in having higher numbers of probiotic bacteria in the end product.

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الجودة الميكروبية للجبنة البيضاء الطرية المنتجة بشكل تقليدي في الأردن ودراسة استعمالها في إنتاج جبنة بيضاء طرية غنية بالمحفزات الحيوية (جبنة بروبيوتيك)

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ملخـــــــــــــــــــص

لتحديد الجودة الميكروبية للأجبان البيضاء الطرية تم القيام بجمع 30 عينة من الجبنة البيضاء الطرية من معامل ومصانع الألبان في المحافظات الرئيسية في الأردن. شمل الفحص كذلك إجراء بعض الفحوصات الكيماوية والفيزيائية على العينات التي تم جمعها. وتمت تعبئة استبانة خاصة بالسلامة والجودة والتصنيع في معامل ومصانع الألبان وذلك من خلال مقابلة الأشخاص أصحاب العلاقة.

كانت معدلات أعداد الطبق القياسي واعداد كل من بكتيريا حمض اللبن والبكتيريا المعوية والخمائروالأعفان 8.3 , 7.9, 5.4، 3.0 لوغريتم وحدة مكونة للمستعمرات /غم، على التوالي. كان هناك ارتباط معنوي موجب (0.90) بين عدد الطبق القياسي وعدد بكتيريا حمض اللبن, الذي يدل على أن معظم البكتيريا في العينات هي بكتيريا حمض اللبن. كان عدد بكتيريا *ستافيلوكوكس اوريس* أقل من 10 وحدة مكونة للمستعمرات /غم, ولم تحتوي أي من العينات على *سالمونيلا* في 25 غم من الجبنة.

كانت معدلات الرقم الهيدروجيني و نسبة الحموضة (% حامض اللاكتيك) و نسبة الرطوبة و نسبة الملح في المحلول الملحي والجبنة ونسبة الرماد هي: , 0.53%, .12%, 9.4% و 9.5%، على التوالي. أعطت 14 عينة من الـ 30 عينة نتيجة موجبة لفحص الفوسفاتيز، مما يدلل على استخدام حليب غير مبستر في التصنيع. تعكس الأعداد العالية من الميكروبات في عينات الجبنة تدني الظروف الصحية أثناء التصنيع والتخزين وعدم ضبط التبريد

وغياب المعاملات الحرارية الملائمة للتخلص من الأحياء الدقيقة. و أمكن من خلال معلومات الاستبانة الخاصة بمعامل ومصانع الاجبان الاستخلاص أنه لا يوجد طريقة مرجعية معتمدة لإنتاج وحفظ الأجبان البيضاء الطرية في الأردن.

تم إنتاج   أنواع من الأجبان البيضاء الطرية المدعمة ببكتيريا التحفيز الحيوي من حليب الأبقار باستعمال بكتيريا *لاكتوباسلس اسيدوفلس* و *بيفيدوبكتريم لاكتيس*. تم تحضين البكتيريا في حليب خالي الدسم وأضيفت البكتيريا إمّا للحليب المستخدم في التصنيع قبل عملية التجبين أو للخثرة قبل عملية الكبس. بعد عمليات الكبس والتقطيع تم حفظ قطع الجبنة في محلول ملحي تركيزه 7% على درجة حرارة 4ْ م لمدة 21 يوم.

بقيت بكتيريا المحفزات الحيوية نشطة في معظم معاملات (الأنواع) الأجبان السابقة بأعداد فوق 10 وحدة مكونة للمستعمرات/ غم لمدة أسبوع. انخفضت الأعداد على أسبوعين عن 10 وحدة مكونة للمستعمرات/ غم. بقيت كل المعاملات التي تمت فيها الإضافة لبكتيريا المحفزات الحيوية للحليب فوق 10^5 وحدة مكونة للمستعمرات /غم جبنة لنهاية التجربة, بينما انخفضت الأعداد في الأنواع التي تمت الإضافة فيها للخثرة أقل من ذلك. بقي الرقم الهيدروجيني ونسبة الملح في المحلول الملحي والجبنة للمعاملات ثابتاً تقريباً خلال فترة التجربة.

للتغلب على الانخفاض في الأعداد, تمت التوصية بزيادة الجرعة من كل نوع من بكتيريا المحفزات الحيوية وذلك بمضاعفة كمية الحليب خالي الدسم المزروع بهذه البكتيريا. كما وجد أنه من الأفضل لإضافة بكتيريا المحفزات الحيوية للحليب عنه للخثرة, لأن الإضافة للخثرة لم تنتج أعداداً أعلى من الإضافة للحليب. ولأن الإضافة للحليب مباشرة أسهل وتوفر الوقت والجهد وأكثر نظافة وتضمن توزيعاً أفضل للبكتيريا في الحليب.

كانت كل أنواع الأجبان الطرية المنتجة في هذه الدراسة مقبولة من الناحية الحسية و الجبنة المنتجة باستعمال بكتيريا *بيفيدوبكتريم لاكتيس* في الحليب هي الأفضل قبولاً، يليها الجبنة المنتجة من خليط بين *لاكتوباسلس اسيدوفلس* و *بيفيدوبكتريم لاكتيس* ومن ثم الجبنة المرجعية. بينما وجد أن الجبنة المنتجة باستعمال خليط من *لاكتوباسلس اسيدوفلس* و *بيفيدوبكتريم لاكتيس* في الخثرة كانت الأقل قبولاً من الناحية الحسية ولكنها لم ترفض من قبل المقيمين.